The use of growth/no-growth models as a tool to predict bread shelf-life

Els Debonne, Ghent University

Ede, 28 January 2020

TABLE OF CONTENTS

INTRODUCTION

Is it safe to eat mouldy bread?

***Penicillium* spp. *Cladosporium* spp. *Aspergillus* spp.**

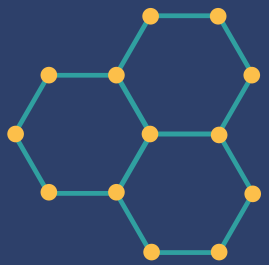
…

visible mycelia → natural repellant

invisible network → breathing problems and allergic reactions

mycotoxins → diseases and death

chemical preservatives



"Any food that requires enhancing by the use of **chemical substances** should in no way be considered as food."

– JOHN H. TOBE

"Old people shouldn't eat healthy foods. They need all the **preservatives** they can get."

– ROBERT ORBEN

Traditional bread

2 – 5 days
retrogradation

Par-baked bread, toast bread

Clean label, MAP → 3 weeks
With preservatives, MAP → 6 - 8 weeks

LITERATURE REVIEW

Sourdough

Antifungal compounds (chemicals):
organic acids; acetic acid, lactic acid, phenyllactic acid, …
pH dependent antifungal effect
C_{TOT} and pH

Essential oils & plant extracts

Natural character
Chemical (volatile) compounds
Strong sensorial and physico-chemical adverse effects
Antifungal = anti – fungi (moulds AND yeasts)

Active concentration expressed on the aqueous phase

Micro-organisms are only **active** in the aqueous phase.
Migration of antifungal compounds (water versus oil phase)

Protonated form of **organic acid** (undissociated concentration).
➔ **Undissociated acid** (mmol) / L aqueous phase ➔ **C_{HA} (mM)**

STUDY OBJECTIVES

Screening

Natural antifungal compounds

G/NG models

Data analysis

Shelf-life

Bread baking trials & storage

METHODOLOGY

Chemicals

Screening of antifungal activity requires **either** working with **standardized** amounts of pure chemicals **OR** requires **detection** methods of chemicals in food products

In-vitro screening

Micro versus **macro** dilution

Selection of **growth medium**

G/NG models + validation in

Development of models

Validation with bread shelf-life

METHODOLOGY

chemicals

Sourdough – organic acids

pH effect on growth of moulds?

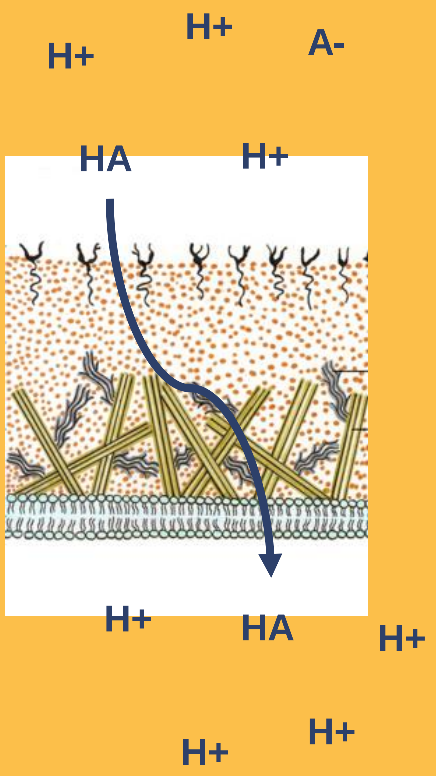
Weak organic acids

- Acetic acid
- Lactic acid
- Phenyllactic acid

- Undissociated acid (C_{HA})

- Henderson- Hasselbalch equation

$$pH = pK_a + \log_{10} \frac{[A^-]}{[HA]}$$



METHODOLOGY

chemicals

Sourdough – organic acids

pH effect on growth of moulds?

Weak organic acids

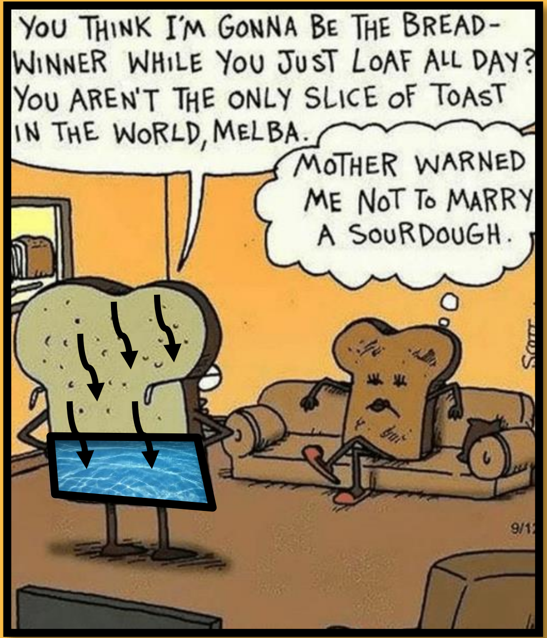
- Acetic acid
- Lactic acid
- Phenyllactic acid

- Undissociated acid (C_{HA})

- Henderson- Hasselbalch equation

$$pH = pK_a + \log_{10}\frac{[A^-]}{[HA]}$$

- C_{HA} in mmole / L aqueous phase



Example:
33 % moisture
active concentration = 3 x conc

Essential oils – terpenes, terpenoids, phenylpropenes & others

METHODOLOGY

chemicals

Lipophilic behavior of EOs/ components

Partitioning to oil – water phase

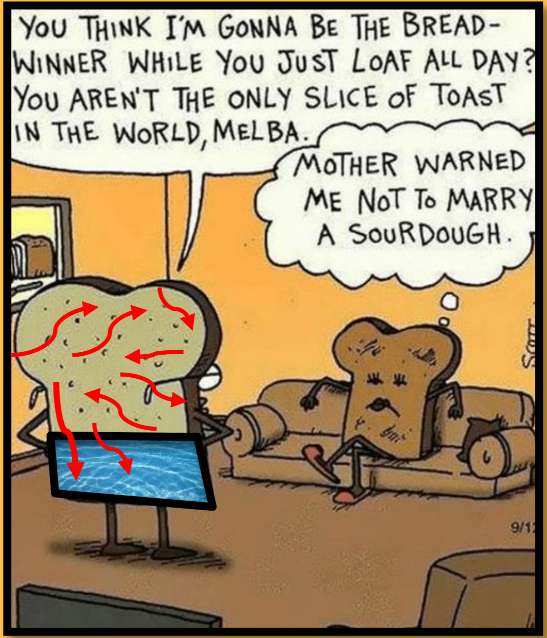
- Kp: partitioning coefficient

- Modified Henderson- Hasselbalch equation

- e.g. thyme essential oil (thymol)

$$C_{thymol,\,aqua} = \frac{n_{TOT,thymol}}{m_{TOT} * \left(K_p * \frac{r}{\rho_{oil}} + \frac{1-r}{\rho_{aqua}}\right)}$$

- C_{aqua} in mmole / L aqueous phase



Example thyme EO (~ thymol):
33 % moisture
Kp (thymol) = 3,34 ($10^{3,34}$/1: parts oil/water)
Oil in bread: 57% (free) of 1,2% lipids in flour

METHODOLOGY

In-vitro screening

Growth of fungi

Screening method can vary.

Important to know the **mode of action** of the chemical compound, e.g. volatile behavior of EOs.

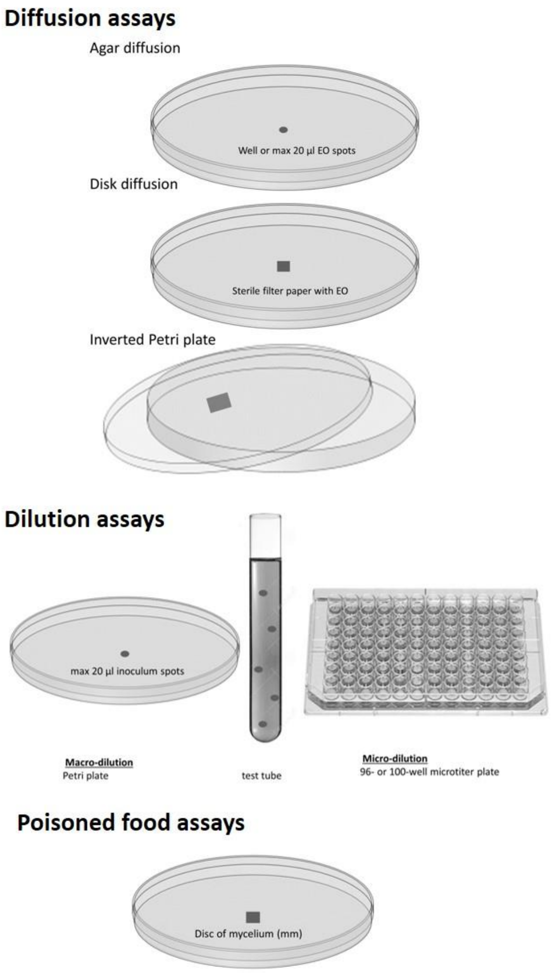
- **Essential oils**

The **chemical variability** of EOs due to variations in **geographical** conditions, **age** of the plants, time of **harvesting** and the method of **extraction**, complicates the use of EOs as natural preservatives in food products.

Therefore in-vitro screening requires **standardization** of the chemicals.

- **Organic acids**

Micro-and **macro** dilution methods



METHODOLOGY

In-vitro screening

Growth of fungi

Screening method can vary.

Important to know the **mode of action** of the chemical compound, e.g. volatile behavior of EOs.

- **Essential oils**

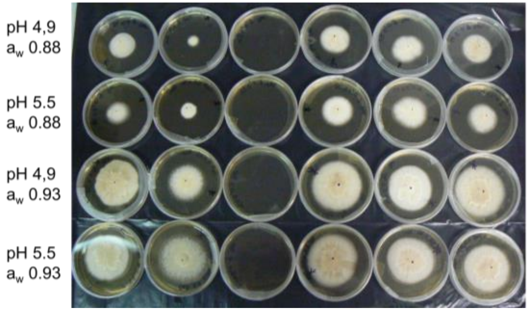
The **chemical variability** of EOs due to variations in **geographical** conditions, **age** of the plants, time of **harvesting** and the method of **extraction**, complicates the use of EOs as natural preservatives in food products.

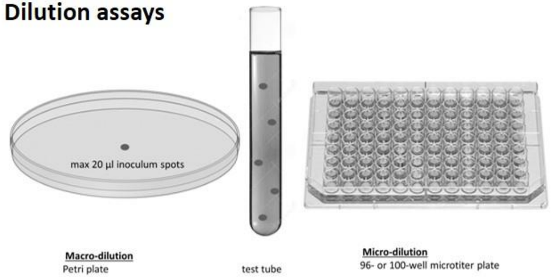
Therefore in-vitro screening requires **standardization** of the chemicals.

- **Organic acids**

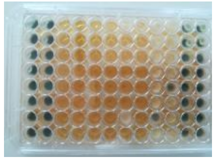
Micro-and **macro** dilution methods

Macro-dilution





Micro-dilution



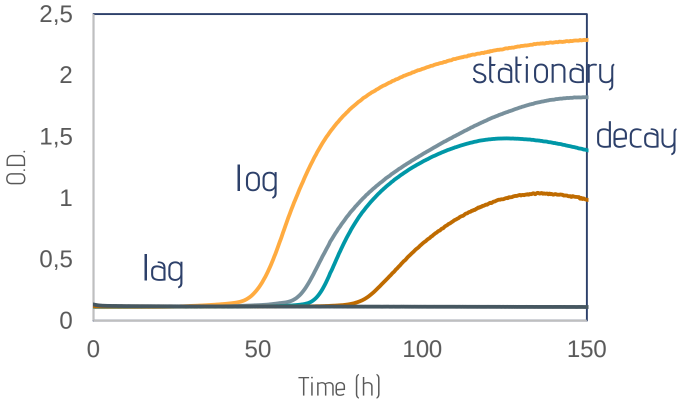
METHODOLOGY

G/NG models

\+

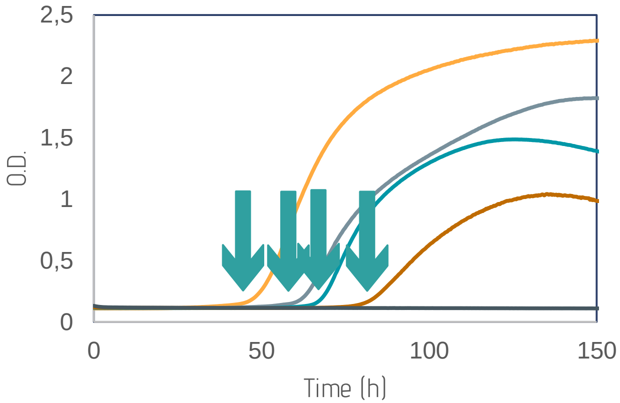
bacteria

Maximal quality levels are defined.



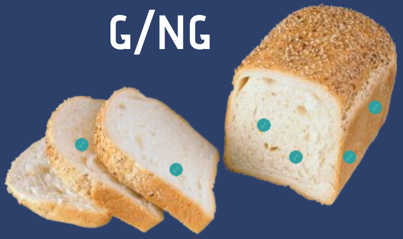
fungi

Growth kinetics is less important.



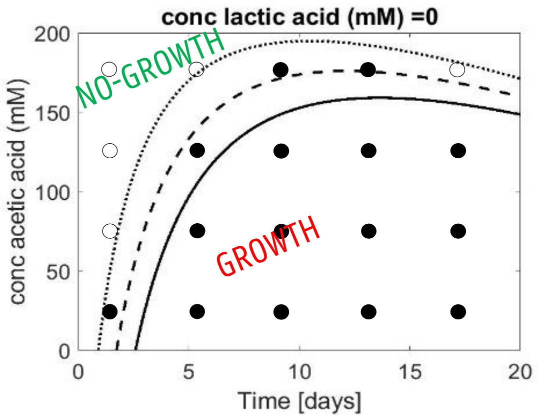
Bread at the end of shelf-life

G/NG

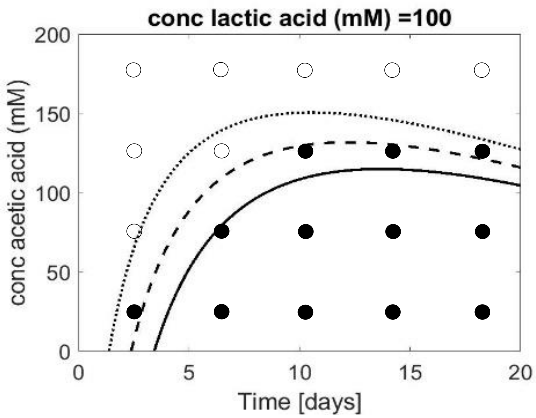


RESULTS ANALYSIS

C_{HA} acetic acid



C_{HA} acetic & lactic acid



G/NG models

Screening method: *macro-dilution*

Mould: *Penicillium paneum*

Incubation temperature: *22 °C*

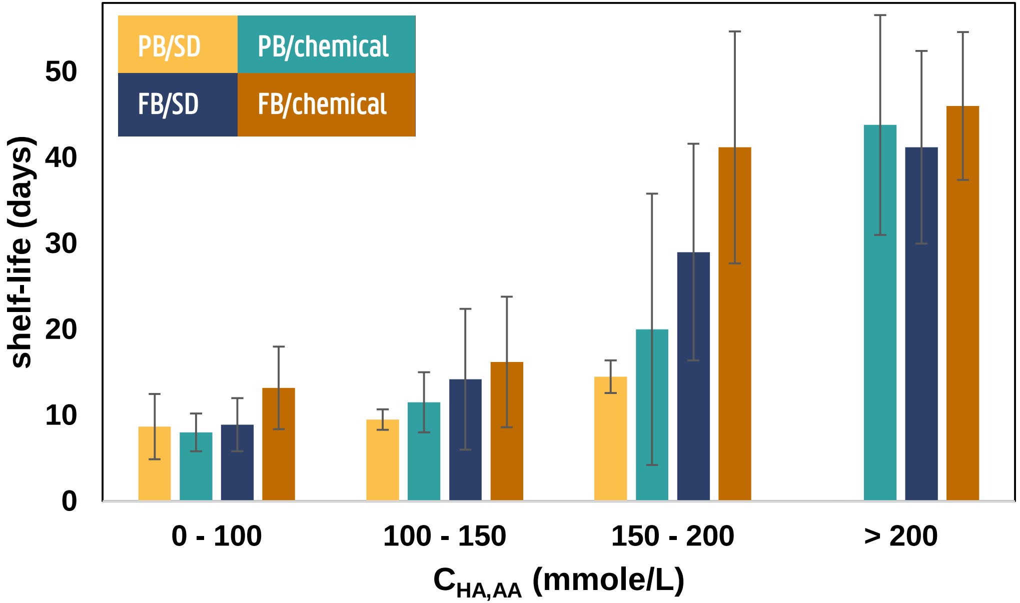
»

Antifungal activity of acetic acid >> lactic acid

C_{HA} acetic acid ≥ 150 – 200 mmole/L

RESULTS ANALYSIS

C_{HA} acetic acid in sourdough bread & in chemically acidified bread



Sourdough with ≥ 150 mM acetic acid:
L. sanfranciscensis
S. cereviseae

Shelf-life

Packaging: *air packaged*
Baking: *par-baked and full-baked*
Contamination: *airborne moulds*
Incubation temperature: *22 °C*

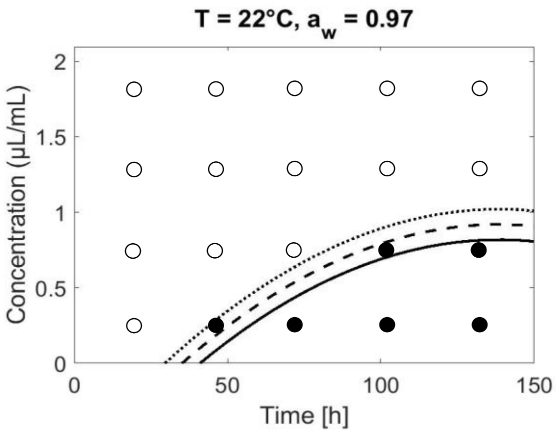
»

- C_{HA} acetic acid ≥ 150 – 200 mmole/L
- No significant difference between SD bread & chem. acid. wheat bread

RESULTS ANALYSIS

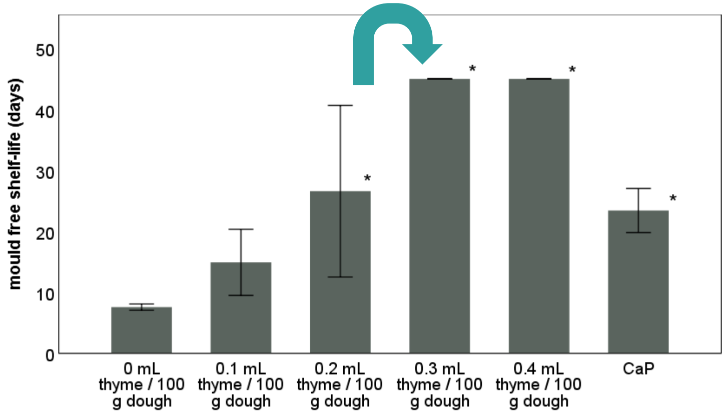
In-vitro screening

of thyme essential oil



Par-baked bread shelf-life

Thyme EO added to bread dough



G/NG models

Screening method: *micro-dilution*
Mould: *Penicillium paneum*
pH: *6* – a_w: *0.97*
Incubation temperature: *22 °C*

»

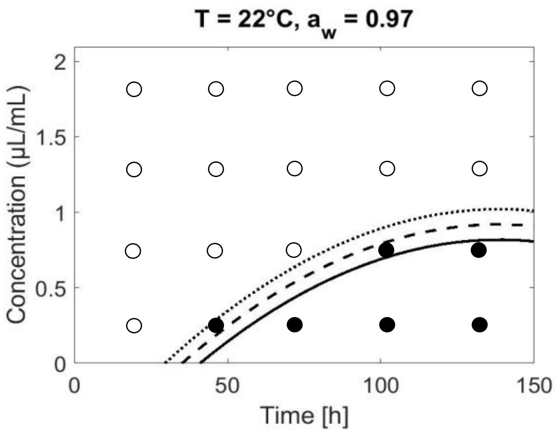
Bread shelf-life

Screening method: *shelf-life*
Moulds: *airborne post-baking contamination*
pH: *6* – a_w: *0.97*
Incubation temperature: *22 °C*

RESULTS ANALYSIS

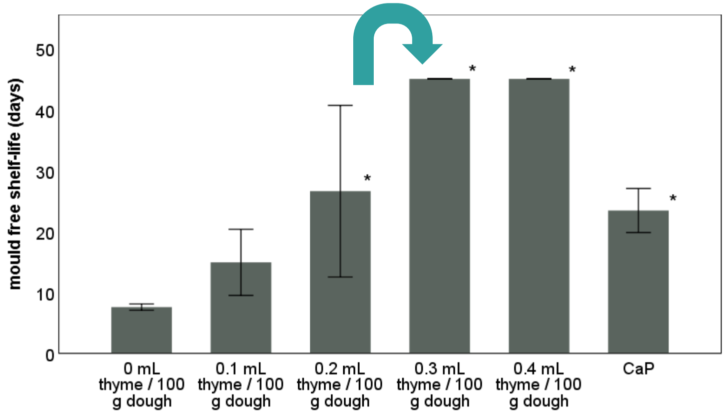
In-vitro screening

of thyme essential oil



Par-baked bread shelf-life

Thyme EO added to bread dough



C (thyme EO) : ± 1 μL / mL medium

»

0.2 – 0.3 mL / 100 g dough

5 – 7 μL / mL aqeous phase in bread (modified HH equation + moisture content of bread)

➔ Further optimization needed

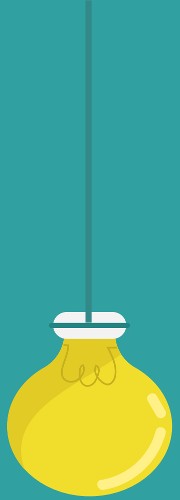
CONCLUSIONS

Take-home messages:

1. Benefits of chemical preservatives (& **E-numbers**)
2. **G/NG models** as a tool to screen antifungal compounds
3. Role of expressing **undissociated** acid concentrations
4. Antifungal effect of sourdough is more than pH alone

And the most important thing: validation of in-vitro G/NG models results in bread products is essential to obtain safe & qualitative food products!

BIBLIOGRAPHY



- DEBONNE (2019). *Growth/no-growth models of in-vitro growth of Penicillium paneum as a function of thyme essential oil, pH, a_w, temperature.* Food Microbiology (Elsevier).
- DEBONNE (2020). *Validation of in-vitro antifungal activity of the fermentation quotient on bread spoilage moulds through growth/no-growth modelling and bread baking trials.* LWT (Elsevier).
- DEBONNE (xxxx). *Comparison of the antifungal effect of undissociated lactic and acetic acid in sourdough bread and in chemically acidified wheat bread.*. Unpublished results

THANKS

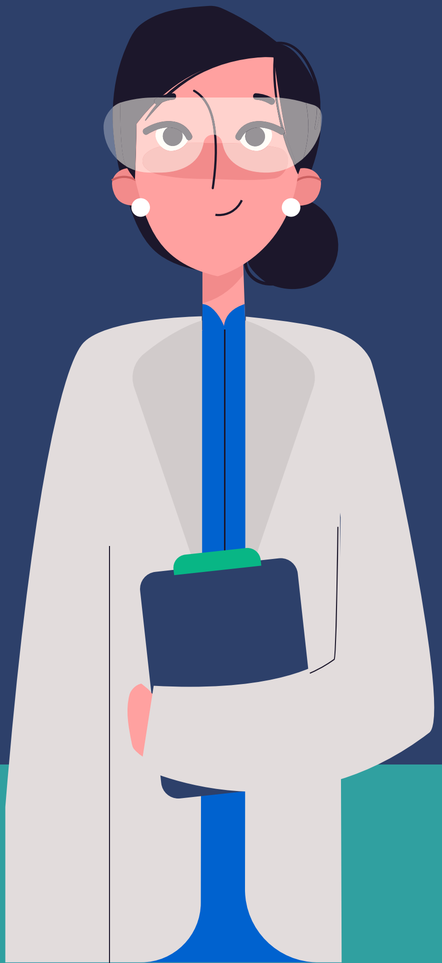
Does anyone have any questions?

Els.Debonne@ugent.be
+32 9 243 24 94
Ghent University

in

ir. Els Debonne
PhD student/Teaching Assistant

DEPARTMENT OF FOOD TECHNOLOGY, SAFETY & HEALTH
FACULTY OF BIOSCIENCE ENGINEERING
GHENT UNIVERSITY, BELGIUM



CREDITS

Ph.D promotors

- Prof. dr. ir. Frank Devlieghere (Ghent University)
- Prof. dr. ir. Mia Eeckhout (Ghent University)
- Prof. dr. ir. Filip Van Bockstaele (Ghent University)

Presentation template & features:

- Presentation template by Slidesgo
- Author introduction slide photo created by Freepik