



**HAL**  
open science

## DEVELOPMENT OF NEW HEALTH INGREDIENTS FROM TURMERIC FERMENTATION

Morgan Le Rouzic, Francois Krier, Fabrice Coloma, Vincent Phalip, Rozenn Ravallec, François Coutte

► **To cite this version:**

Morgan Le Rouzic, Francois Krier, Fabrice Coloma, Vincent Phalip, Rozenn Ravallec, et al.. DEVELOPMENT OF NEW HEALTH INGREDIENTS FROM TURMERIC FERMENTATION. BIOKET, BioEconomy For Change, May 2023, Trois-Rivières - QUEBEC, Canada. hal-04551900

**HAL Id: hal-04551900**

**<https://hal.science/hal-04551900>**

Submitted on 18 Apr 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# DEVELOPMENT OF NEW HEALTH INGREDIENTS FROM TURMERIC FERMENTATION



Morgan Le Rouzic<sup>1,2</sup>, François Krier<sup>1</sup>, Fabrice Coloma<sup>2</sup>, Vincent Phalip<sup>1</sup>, Rozenn Ravallec<sup>1</sup>, François Coutte<sup>1</sup>

<sup>1</sup> UMRt BioEcoAgro laboratory – <sup>2</sup> EURABIOTECH company



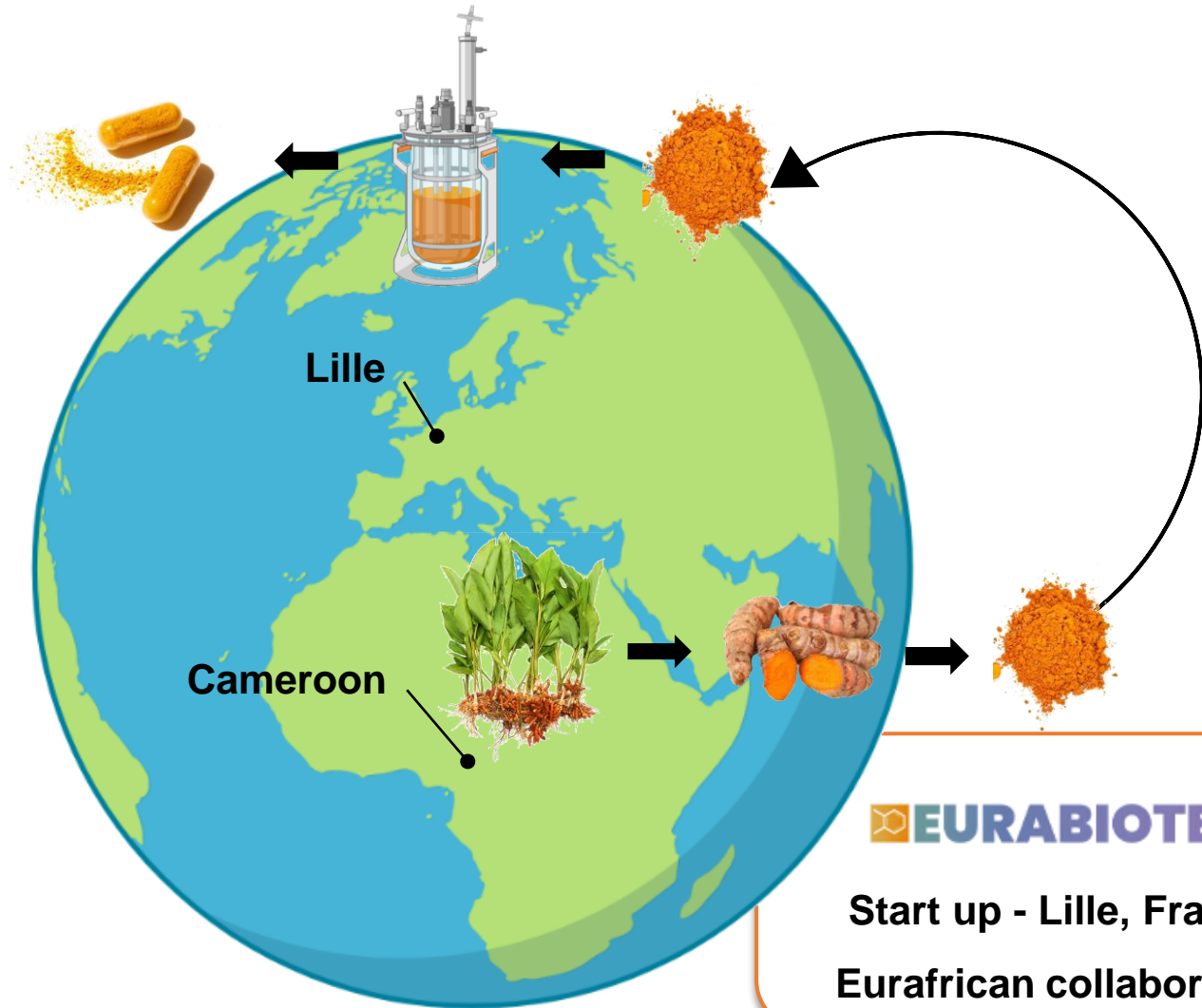
Innovative turmeric extracts



High titration in active molecules



Increased health effects



**EURABIOTECH**  
Start up - Lille, France  
Eurafrican collaboration

# Curcuma longa



Family: *Zingiberaceae*      94 different species

(Theplantlist.org)



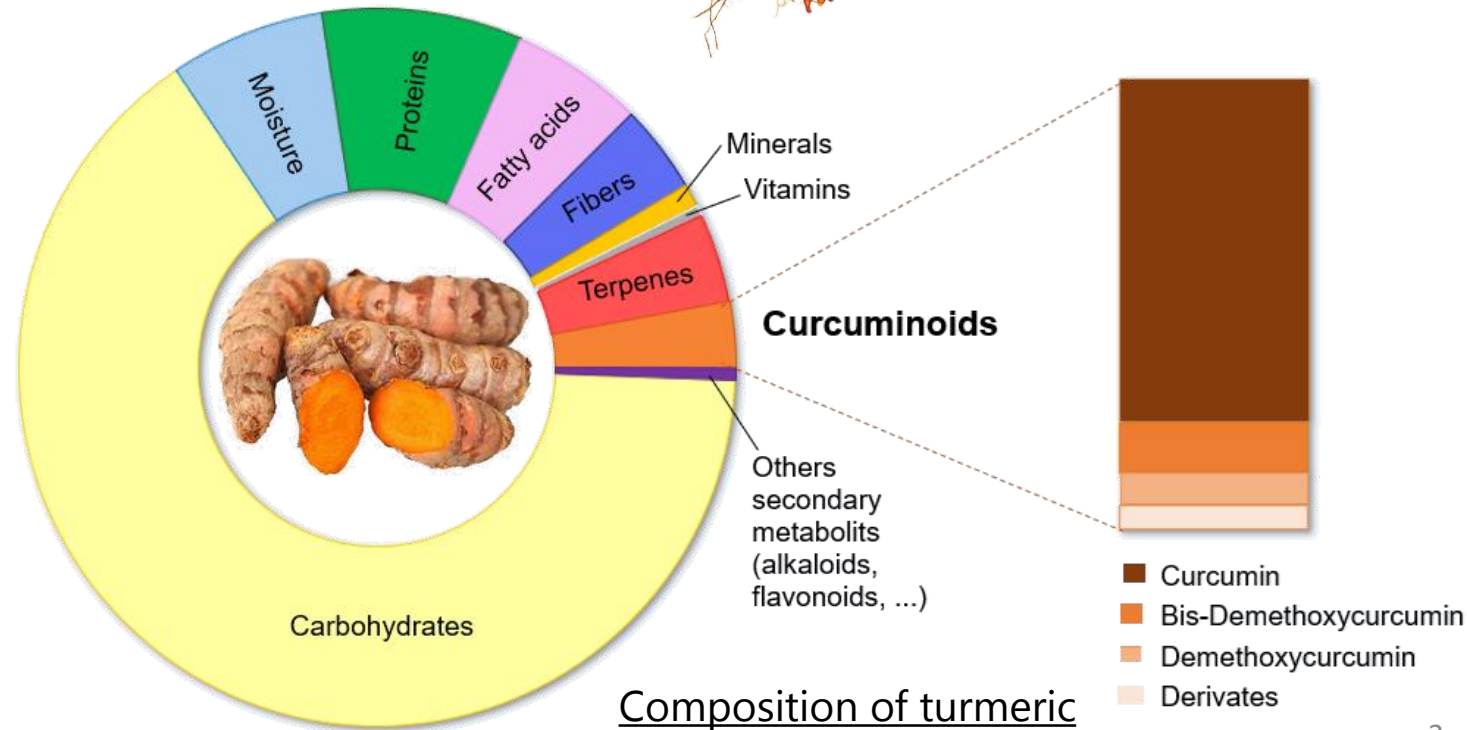
Origin : Asia (Southeast)

Cultivation : Asia, **Africa**, South America



World distribution of turmeric production

(Encyclopedia of life)



Composition of turmeric

(Ikpeama *et al.*, 2014 ; Zhang & Kitts 2021)



# Why *Curcuma longa* ?

## Uses since 6000 years (FAO)

Gastro-intestinal system

Traditional use (EMA, WHO)

Phytotherapy use (ESCOP)

↗ Pharmaceuticals, cosmetics,

## Nutraceuticals

→ **Anti-inflammatory**

→ **Antioxydant**

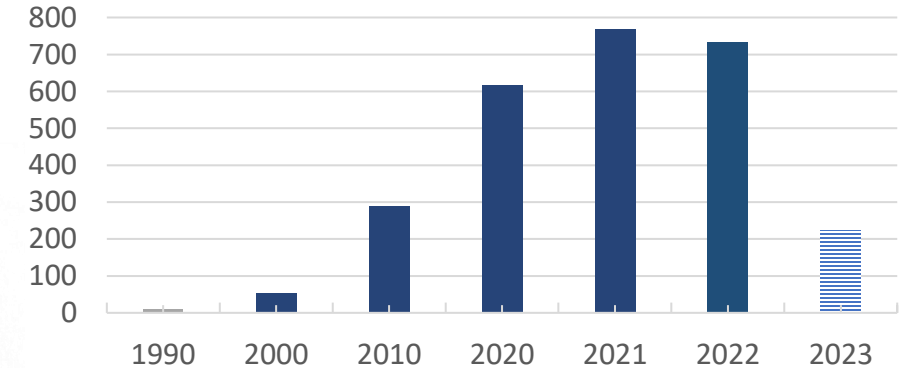
→ **Anti-cancer**

→ **Anti-diabetic**

→ **Antibacterial**

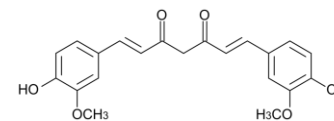


Number of publications

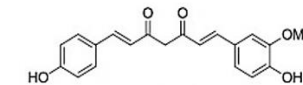


## Scientific publications about turmeric per year

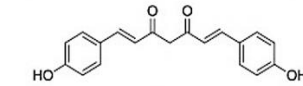
(PubMed, Key word : turmeric)



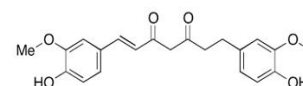
Curcumin



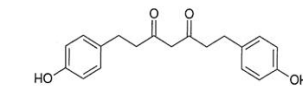
Demethoxycurcumin



Bis-demethoxycurcumin



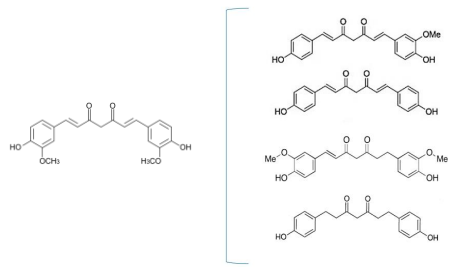
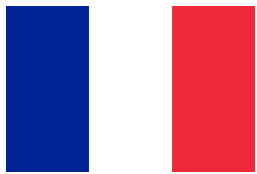
Dihydrocurcumin



Tetrahydrocurcumin

## Molecular representation of curcumin and some of its derivatives

# Regulatory context of supplements



**Commission européenne**      **EFSA**  
EUROPEAN FOOD SAFETY AUTHORITY

**DG CCRF**  
Direction générale de la concurrence,  
de la consommation et de la répression des fraudes

**Declaration of marketing for supplements**

**anses**

**Maximum Daily Dose :**  
Curcumin: 180 mg/j  
Tetrahydrocurcumins: 140 mg/j

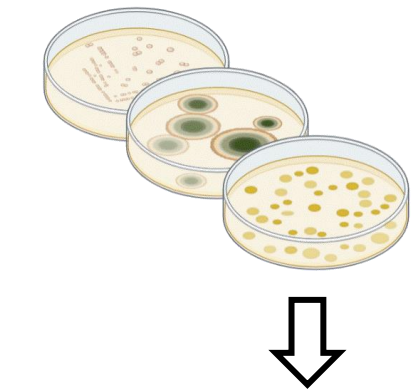
**Food and Drugs Act**

Natural Health Products Regulations

- Natural and Non-prescription Health Products Directorate
- Marketed Health Products Directorate
- Health Products and Food Branch Inspectorate

**Product and site licences**

# Why ferment turmeric ?



**Lactic acid bacteria** 1, 2, 3, 4, 5, 14

**Aspergillus sp.** 6, 7, 8, 9, 10, 15

**Trichoderma sp.** 11

**Rhizopus sp.** 12, 13

**Anti-inflammatory**  
3, 4, 12, 13

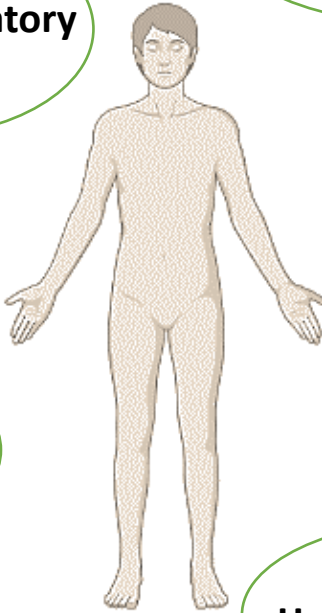
**Antioxydant**  
1, 2, 6, 7, 11, 12, 13, 14

**Anti-obesity**  
9, 13

**Probiotic**  
5

**Hepato-protective**  
8, 9, 15

**Anti-cancer**  
13



1 Pianpumepong *et al.*, 2010, 2 Yong *et al.*, 2023 ; 3 Kim *et al.* 2011 ; 4 Yong *et al.*, 2019 ; 5 Singhal *et al.*, 2016 ; 6 Choi *et al.*, 2013 ; 7 Kim *et al.*, 2014 ;

8 Kim *et al.*, 2014 ; 9 Kang *et al.*, 2015 ; 10 Sulasiyah *et al.*, 2018 ; 11 Mohamed *et al.*, 2016 ; 12 Kim *et al.* 2017 ; 13 Lim *et al.* 2022 ; 14 Lu *et al.* 2023 ; 15 Lee *et al.* 2022

# Project's strategy

## 1) Characterization



Isolation & Identification

*C. longa's* micro-organisms



Bioactive molecules

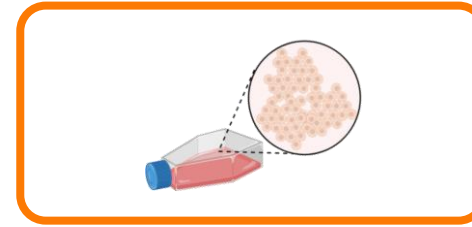
(Qualitative & quantitative)

## 2) Fermentation process development



Fermentation assays

## 3) Bioactivity assays



Cytotoxicity

Anti-inflammatory assays



Antioxydant assays

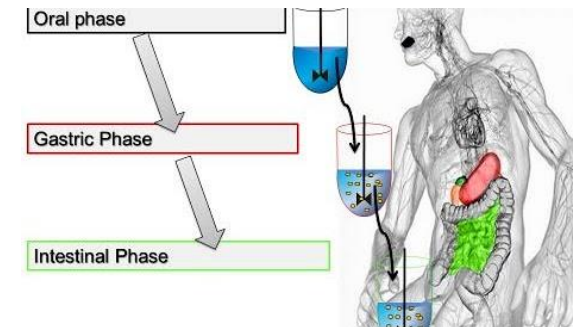


In vivo assays

## 4) Prototype



Simulated gastrointestinal digestion



→ INFOGEST *In Vitro* Model

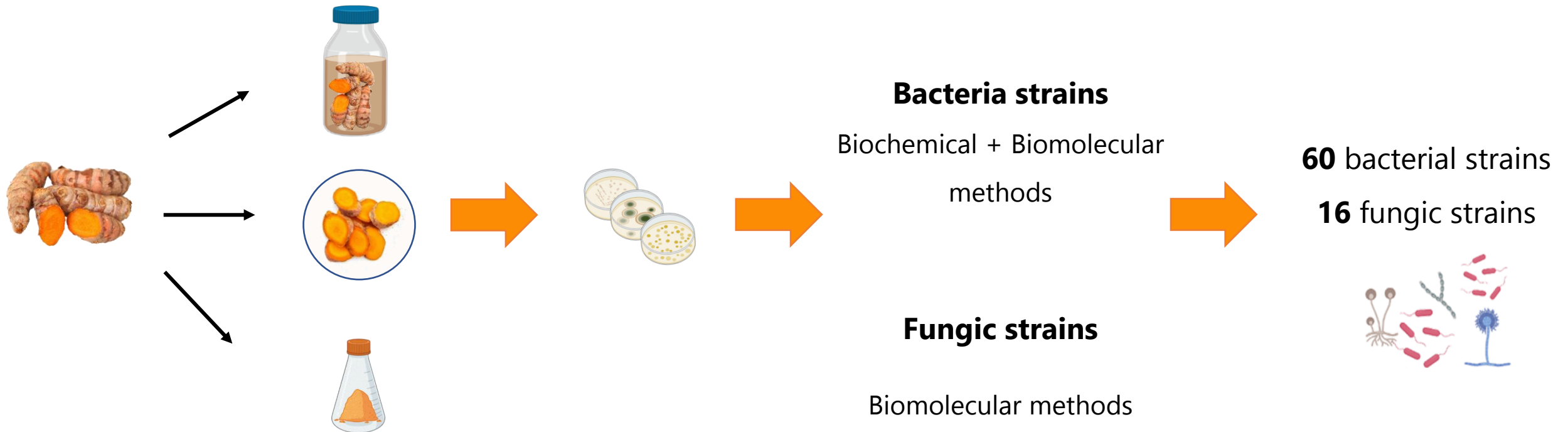
(Minekus *et al.*, 2014)



# 1- Strain selection criteria

## Isolation

## Identification



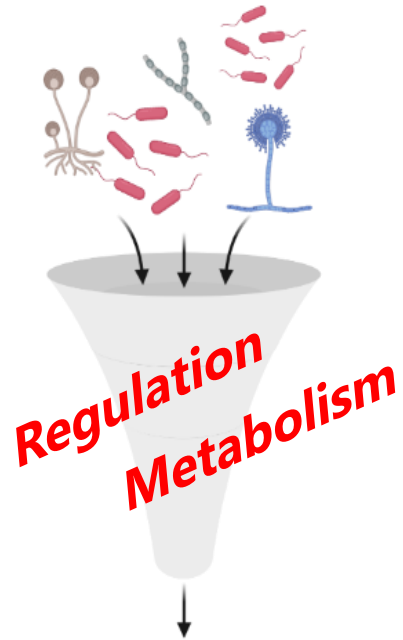
# 1- Strain selection criteria

→ Minimal inhibitory concentrations (MICs)

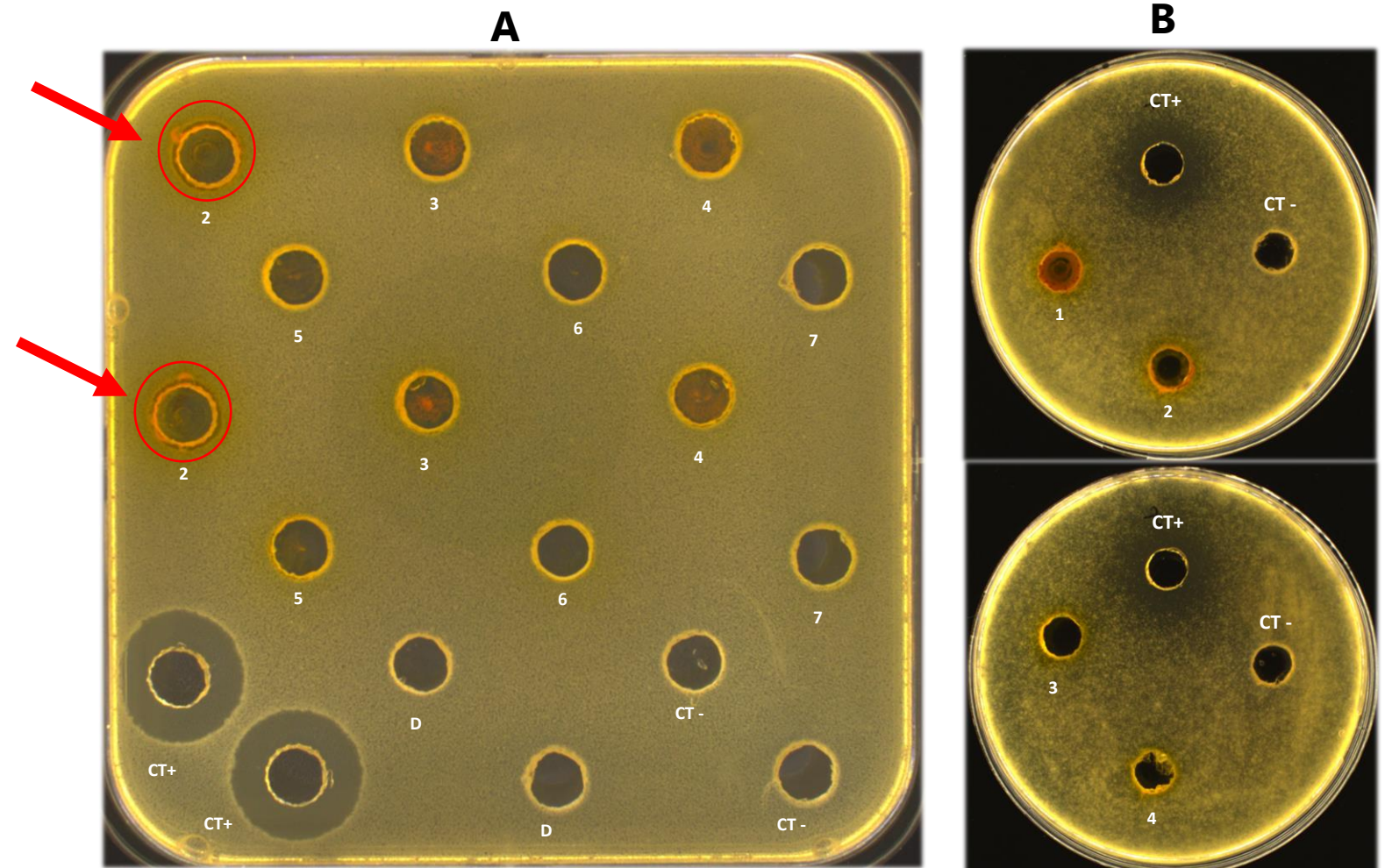
## Screening

60 bacteria strains

16 fungic strains



Candidate strains



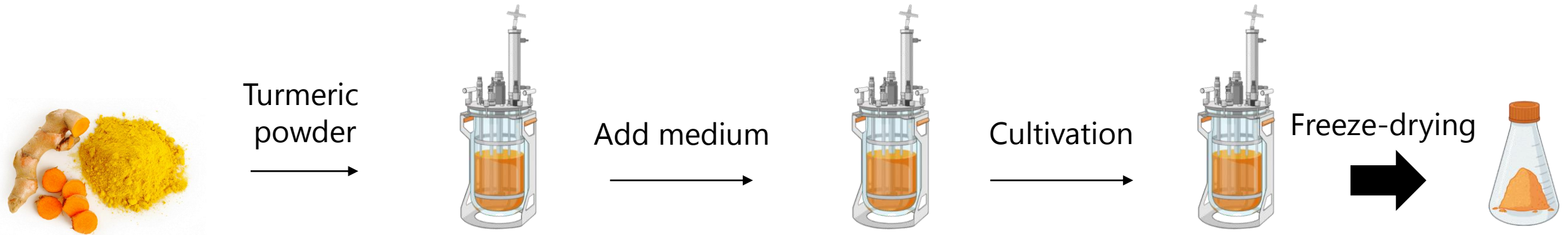
MICs of curcumin against a **bacterial strain (A)** and **fungic strain (B)** isolated from turmeric

Curcumin : (1) 2 mg/ml ; (2) 1 mg/ml ; (3) 0,5 mg/ml ; (4) 0,25 mg/ml ; (5) 0,125 mg/ml ; (6) 0,062 mg/ml ; (7) 0,031 mg/ml

Positive control (CT +): (A) neomycin [30 µg/ml] / (B) cycloheximide [10 µg/ml] ;

DMSO control (D) : DMSO 10% ; Negative control (CT -): physiological water

# 2) Development of turmeric fermentation process

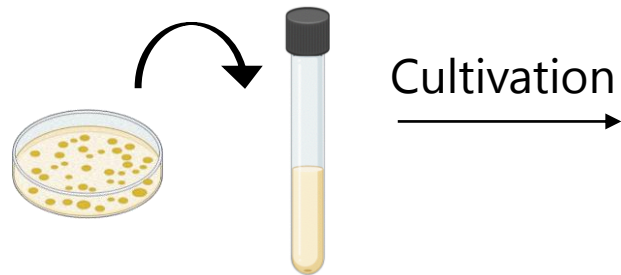


Sterilize  
in contenairs

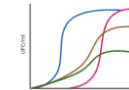
Add medium

Cultivation

Adjust to  
desired density



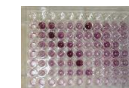
## Analysis



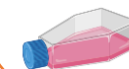
Growth control : Biomass



- Analysis of curcuminoids (HPLC)

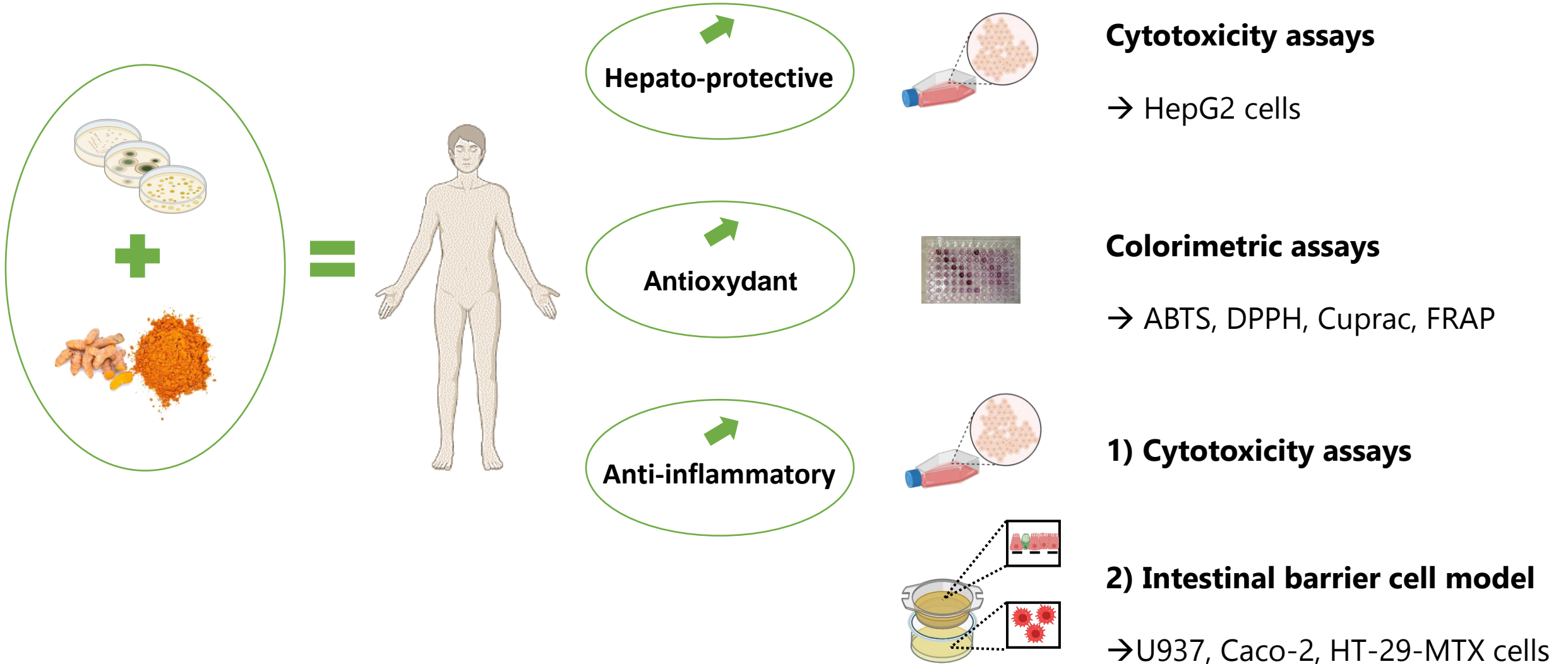


- Antioxidant tests



- Anti-inflammatory tests

# 3) Characterization of bioactivities

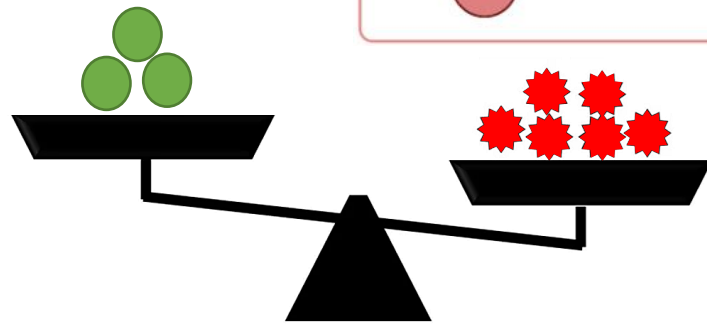
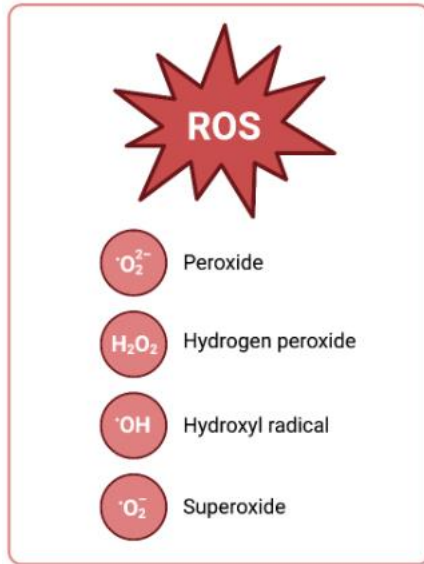




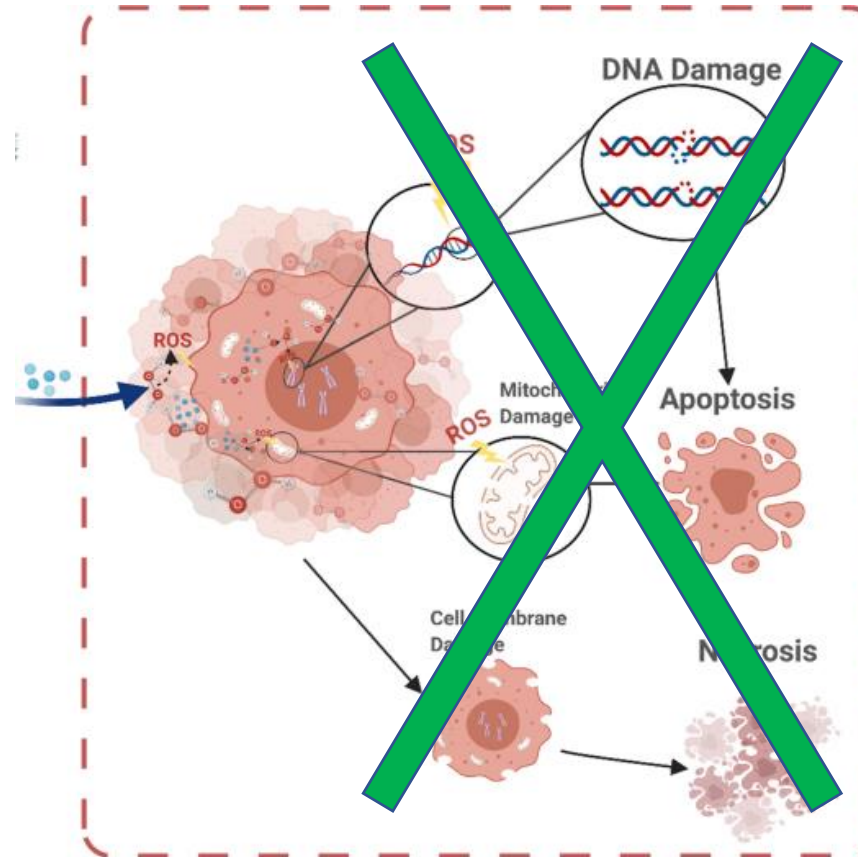
# 3) Antioxydant mechanisms

## Antioxydant

Enzyme, Protein  
Micronutrient  
Polyphenols



Oxydative stress



## Antioxydant mechanisms

- Electron/Proton transfert
- Copper/Iron captation

Cellular repercussions of oxidative stress

Wang *et al.* 2021, Journal of Nanotechnologies

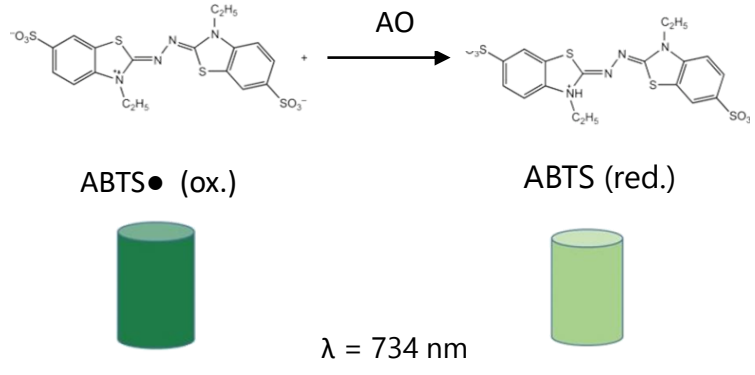


# 3) Antioxydant effect : 4 different mecanismisms

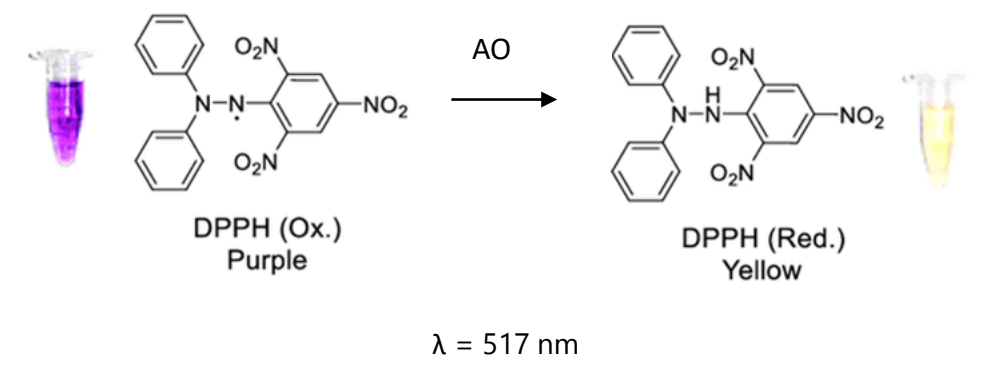
→ Food Industry / Pharmaceutical

Electron / Proton transfert

**ABTS** 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)



**DPPH** 2,2-diphenyl 1-picrylhydrazyl

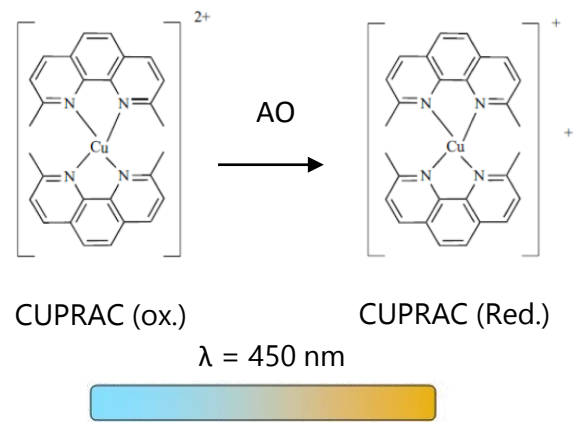


→ Biocontrol

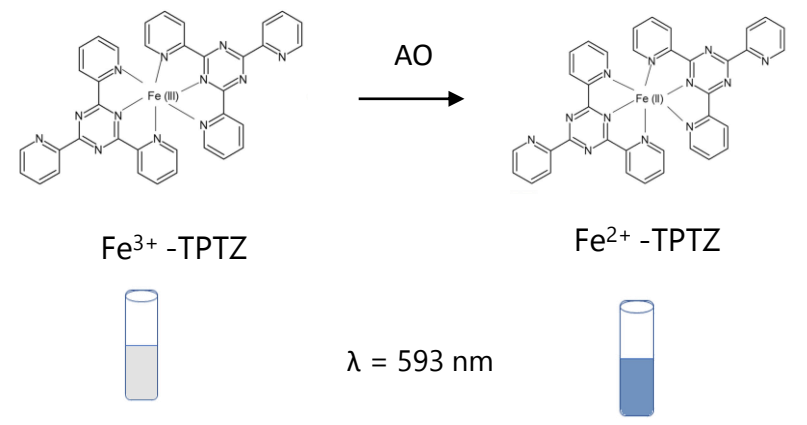
Copper / Iron captation

→ Cosmetology, pharmaceutical

**CUPRAC** Neocuproin (2,9-dimethyl-1,10-phenanthroline)



**FRAP** TPTZ: ferric2,4,6-tripyridyl-s-triazine



# 3) Cascade of intestinal inflammation

Effect of fermented turmeric

↘ **TNF- $\alpha$ , IL-6**

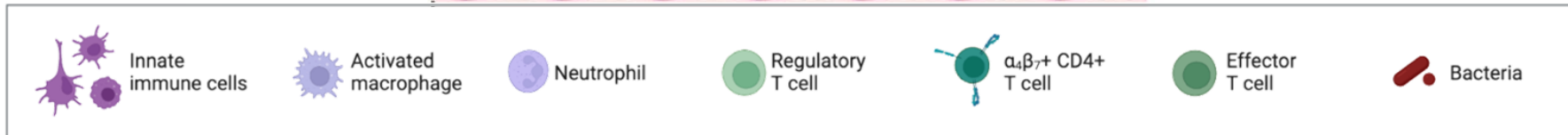
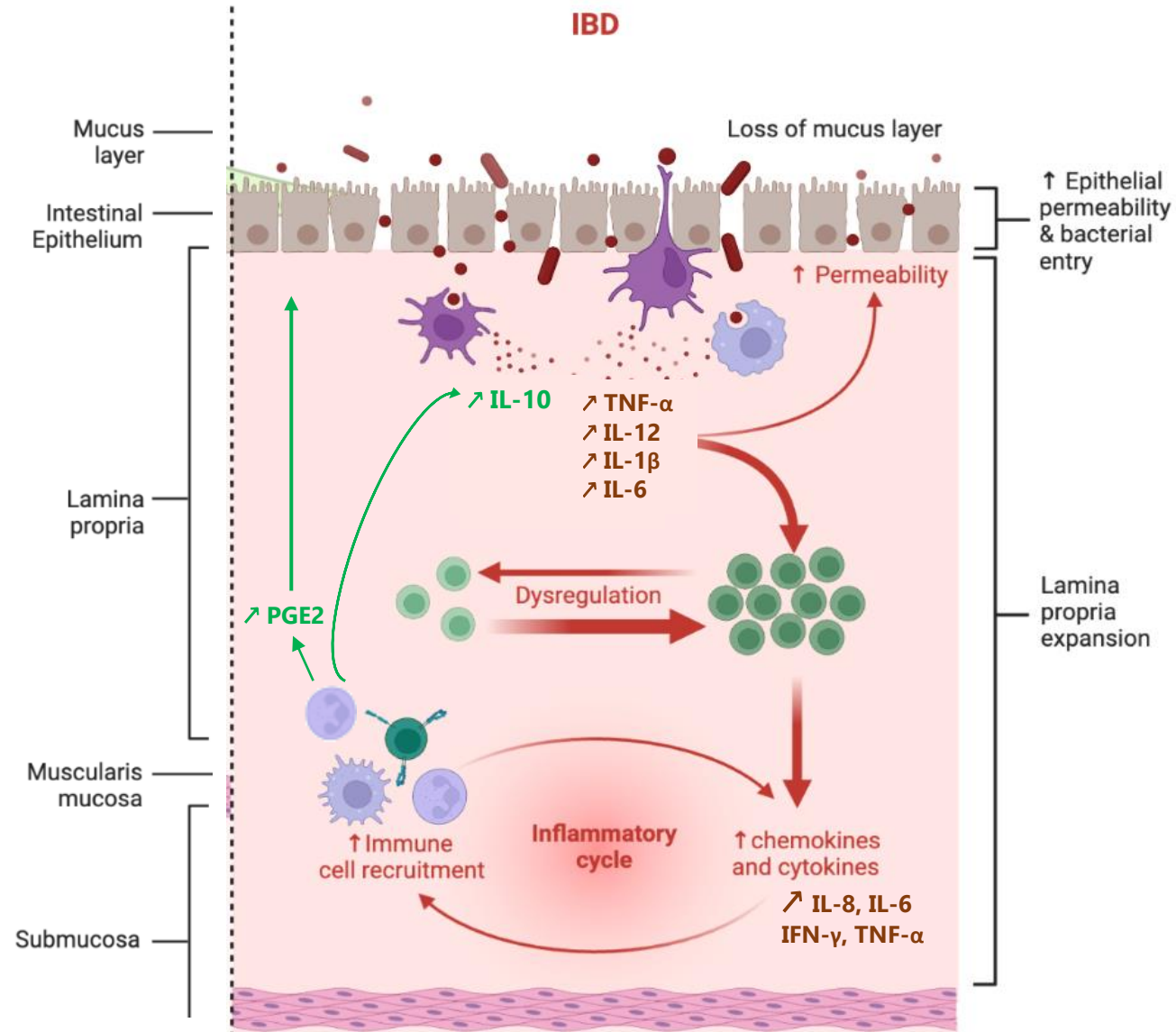
(Tham *et al.* 2010)

↘ **TNF- $\alpha$ , IL-6, PGE2, NF- $\kappa$ B**

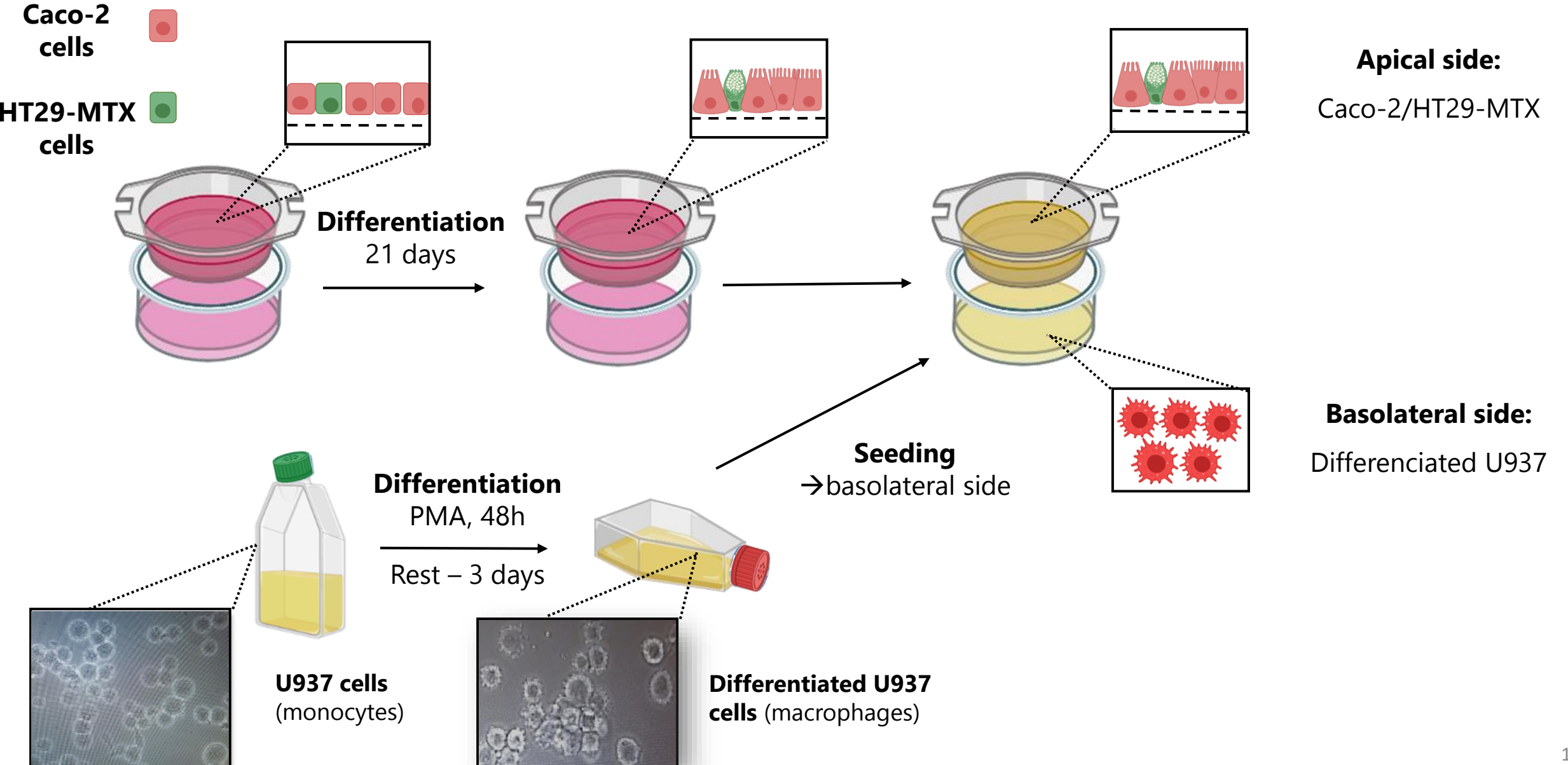
(Kim *et al.* 2017)

↘ **IL-8, IL-12, IL-23**

(Wang *et al.* 2022)

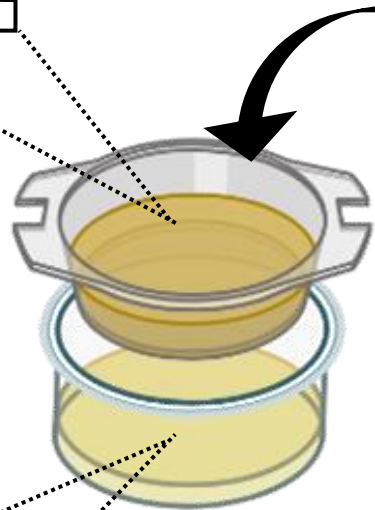
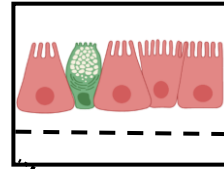


# 3) Anti-Inflammatory effect: Intestinal cell barrier model



# 3) Anti-Inflammatory effect: Intestinal cell barrier model

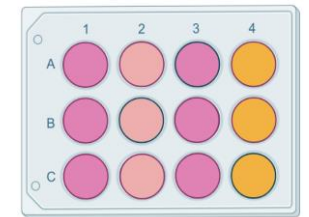
**Apical side:**  
Caco-2/HT29-MTX co-culture



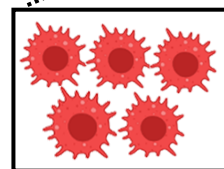
**Apical side:**  
+ Samples  
+ Lipopolysaccharides

Anti-inflammatory control:  
Dexamethason Indomethacin

4h



**Basolateral side:**  
Differentiated U937



## Analysis

Cytokins → TNF-alpha, IL-10, IL-8

Prostaglandin → PGE2



**Luminex®**

# → Take-home Message

**Confidentiality of results**



**Many others bioactivity  
mechanisms**

**Many possible strains**





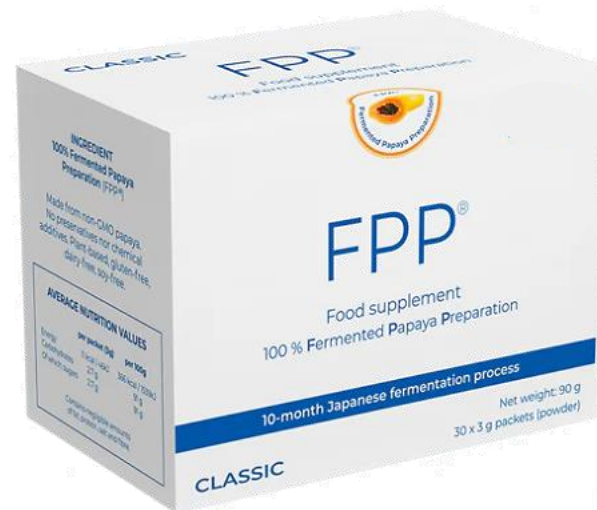
# Fermented Papaya (*Carica papaya* L.)

→ *Saccharomyces* sp.

Patil *et al.* 2021

→ Lactic acid bacteria

Chen *et al.* 2017



**Antioxydant - Immune system – Alzheimer disease – Parkinson - Anemia**

Leitão *et al.* 2022

 EURABIOTECH

**anRT**  
ASSOCIATION NATIONALE  
RECHERCHE TECHNOLOGIE

**MEL**  
MÉTROPOLE  
EUROPÉENNE DE LILLE

BioEcoAgro  



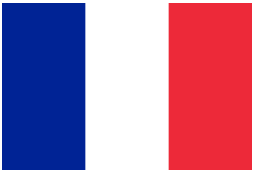

I-SITE  
UNIVERSITÉ  
LILLE NORD-EUROPE

  
CHAIRE  
INDUSTRIELLE  
CHARLES  
VIOLETTE

  
EDSMRE

 Université  
de Lille

# Regulatory context of supplements



Commission européenne  
EFSA  
EUROPEAN FOOD SAFETY AUTHORITY

**Food and Drugs Act**  
Natural Health Products Regulations

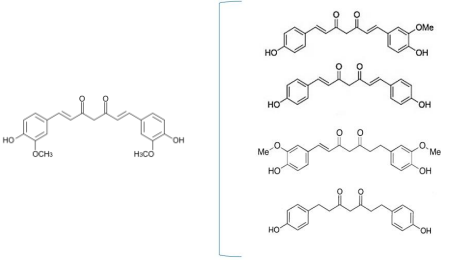
## NOVEL FOODS

“Foods or ingredients whose consumption was negligible or non-existent in the countries of the European Union before **15 May 1997.**”

### Curcumin, Curcuminoids

→ Any process that improves solubility or bioavailability could be subject to the Novel Foods Regulation (EU Novel Food Catalogue).

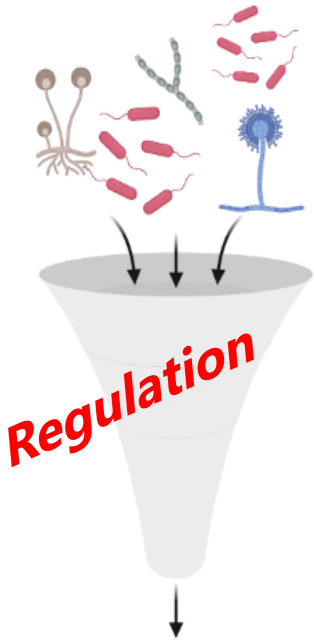
“Foods that have been produced through **new processes**, that do not have a **history of safe** use as a food, or that have been modified by **genetic** manipulation.”



# Regulatory context of supplements

## Screening

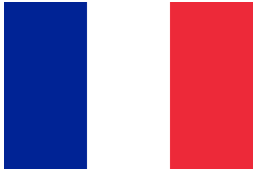
60 bacteria strains  
16 fungic strains



Candidate strains



## Probiotic



Commission européenne  
EFSA EUROPEAN FOOD SAFETY AUTHORITY  
ANSES  
DG CCRF  
Direction générale de la concurrence, de la consommation et de la répression des fraudes

**Health claims authorized**  
**107 – 109 cells/d**  
**Known strains history**

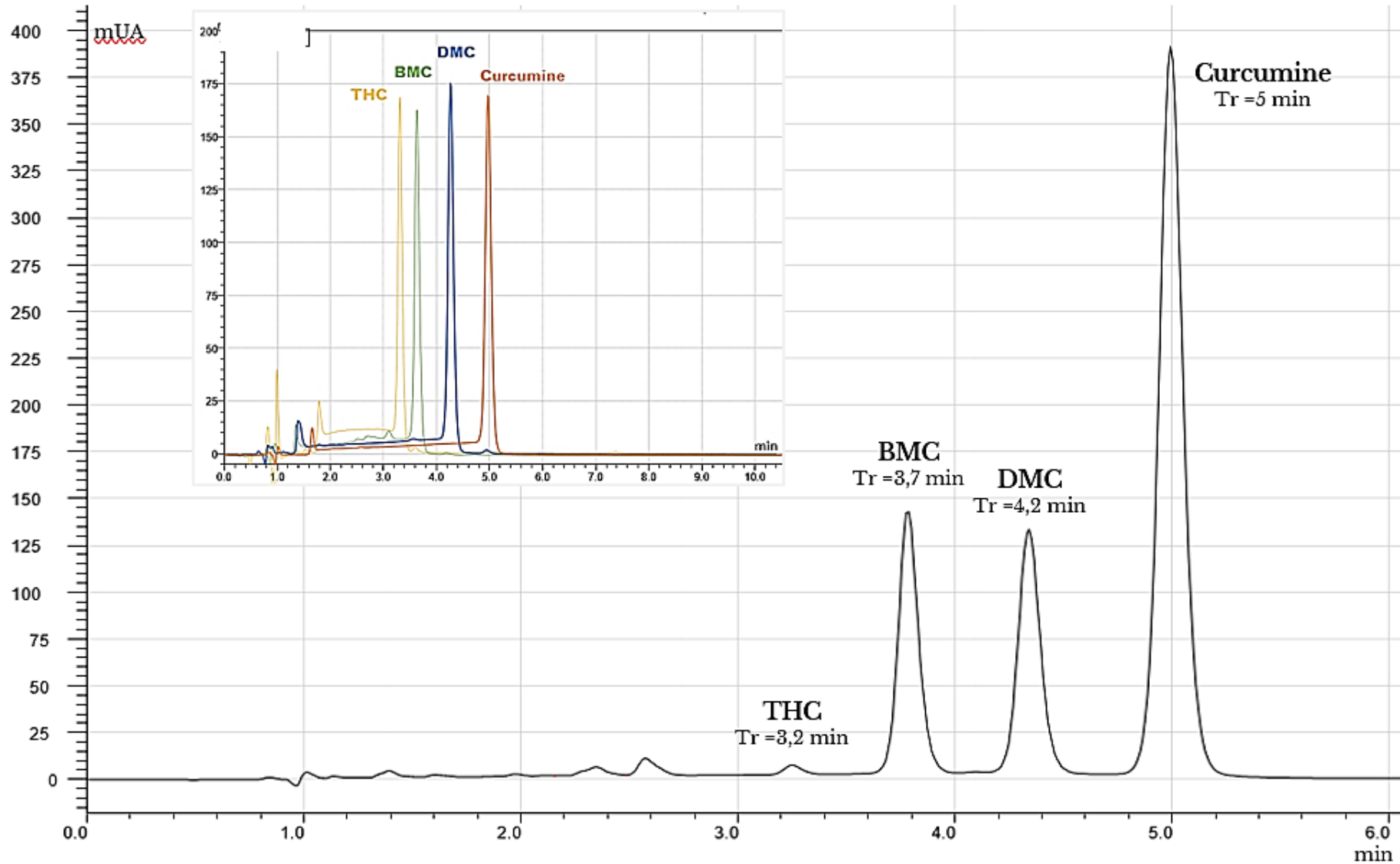
Possible QPS status of safety

**Food and Drugs Act**  
Natural Health Products Regulations  
NNHPD, HPFBI

**Health claims**  
**Posology**  
**Required licences**



# HPLC analysis of turmeric powder



Profil chromatographique de la poudre de curcuma et des standards (HPLC Shimadzu®, colonne PFP, détection-UV 420 nm).  
Curcumine (Tr=5 min), BMC (Tr=3,7 min), DMC (Tr=4,2 min) et THC (Tr=3,2 min).