Enzymatic synthesis of new bioactive compounds endowed with antioxidant and emulsifying activities

Mr. Zied ZARAI

(Assoc. Prof. Dr.-High Institute of Biotechnology-TUNISIA)

University of Sfax
TUNISIA

الجمهورية التونسية
وزارة التعليم العالي والبحث العلمي

Name: ZARAI First name: Zied

- **Associate Professor (Biological engineering), Higher Institute of Biotechnology, University of Sfax**
- **Head of the 'Food Technology' department at the Higher Institute of Biotechnology of Sfax (ISBS).**

TEl :(+216)22061715
E-mail :zied.zarai@isbs.usf.tn

University of Sfax

Université de Sfax
TUNISIE

Sfax

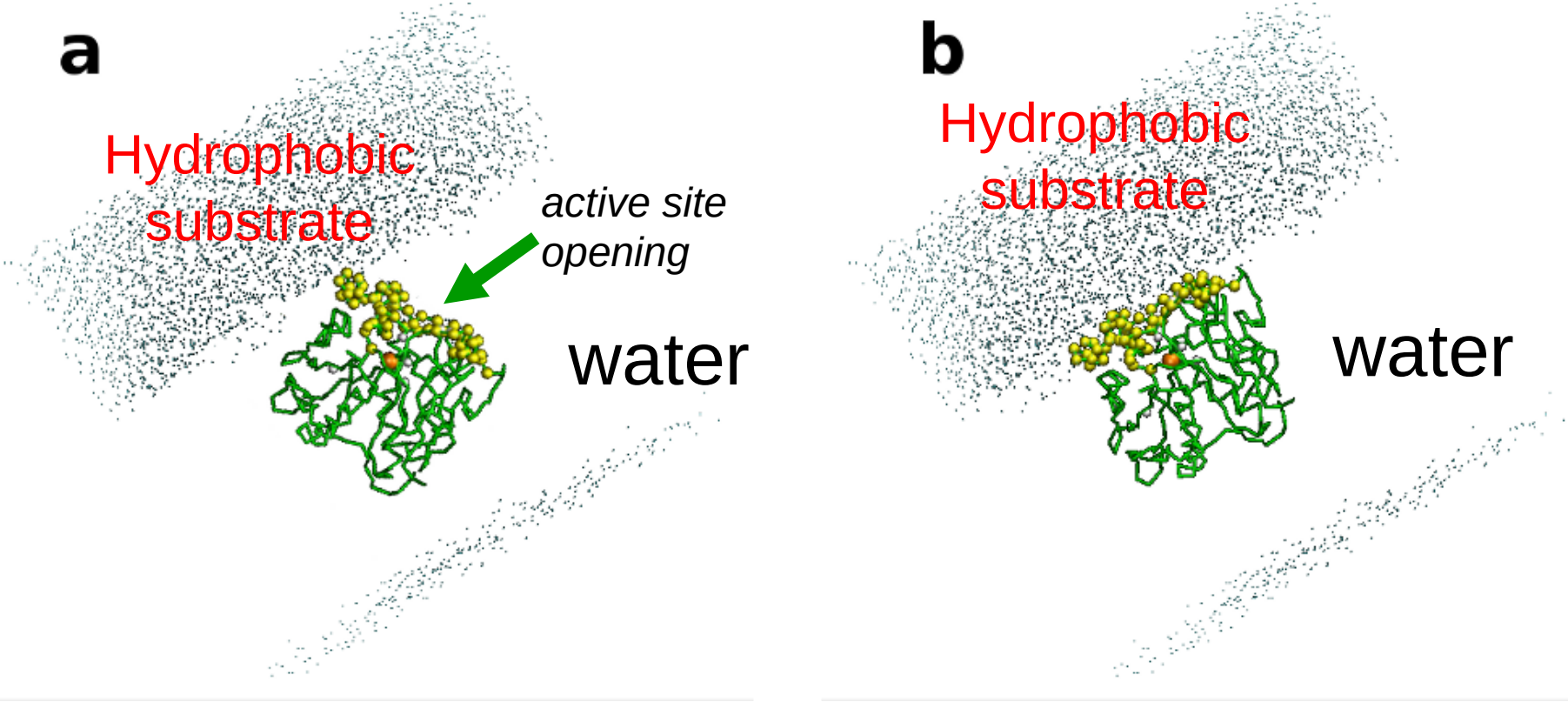
High Institute of Biotechnology, Sfax

- **Adress:** ISBS BP 261, 3038 road of Soukra km 4,5 Sfaxn, Tunisia.
Tel: (216)74 67 43 54
- **Fax:** (216)74 67 43 64
- E-mail : isbs@isbs.rnu.tn
- Site Web:***http://www.isbs.rnu.tn/***

المعهد العالي للبيوتكنولوجيا
بصفاقس

Institut Supérieur de Biotechnologie
de Sfax

Lipases in nature approach and hydrolyze insoluble hydrophobic substrates



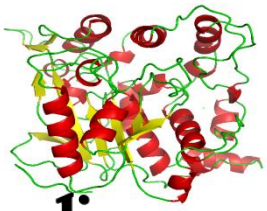
Molecular dynamics simulations of a lipase in a water-oil biphasic system (Martini software)

General Introduction

Valorization of fats

Oil chemistry

Breadmaking



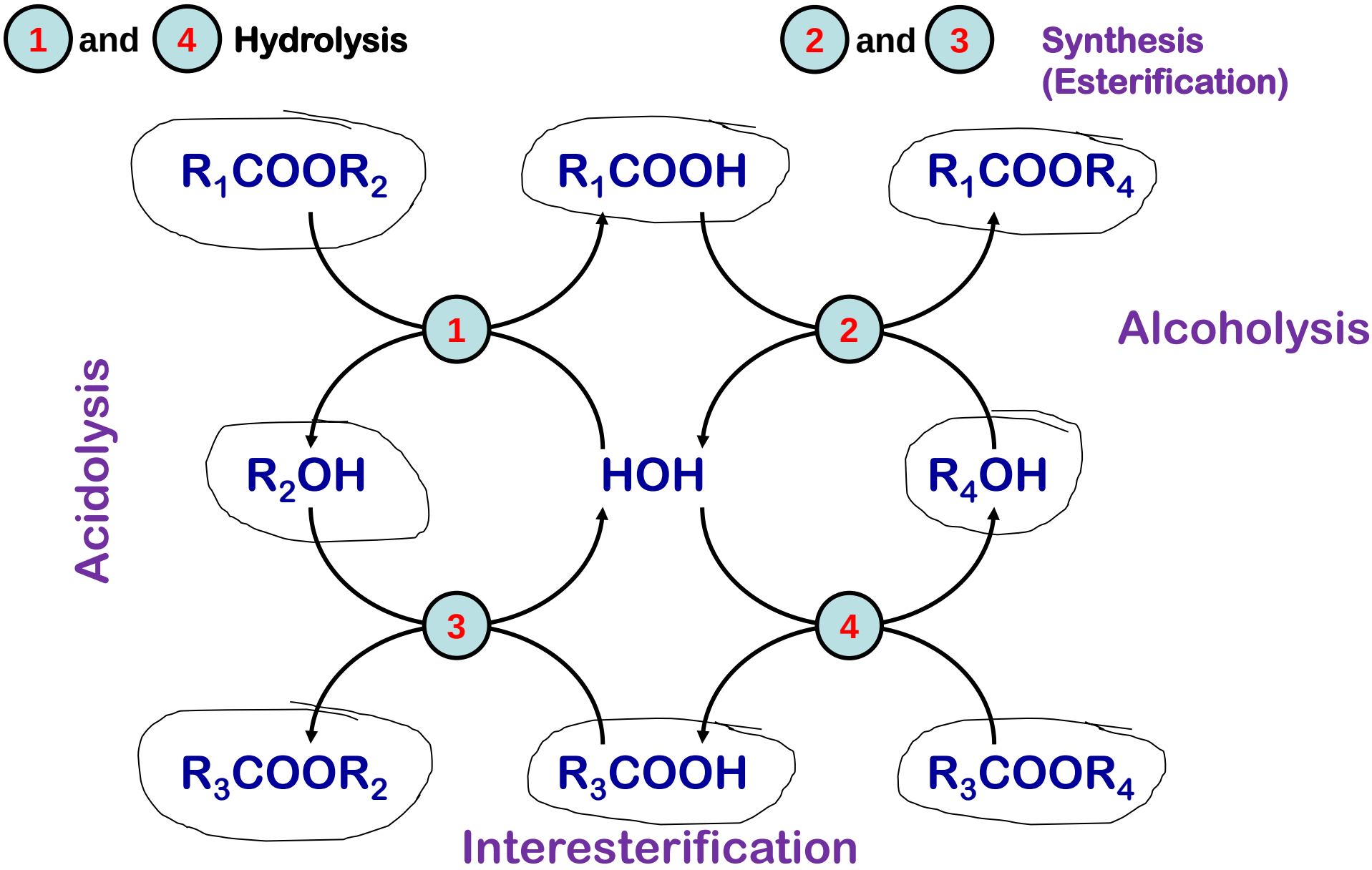
Flavor and aroma

Emulsifying agent

Detergency

Lipases represent around 10% of the global industrial enzyme market and are used in many industrial segments such as detergents, the production of oils and fats, food products and the pharmaceutical sector.

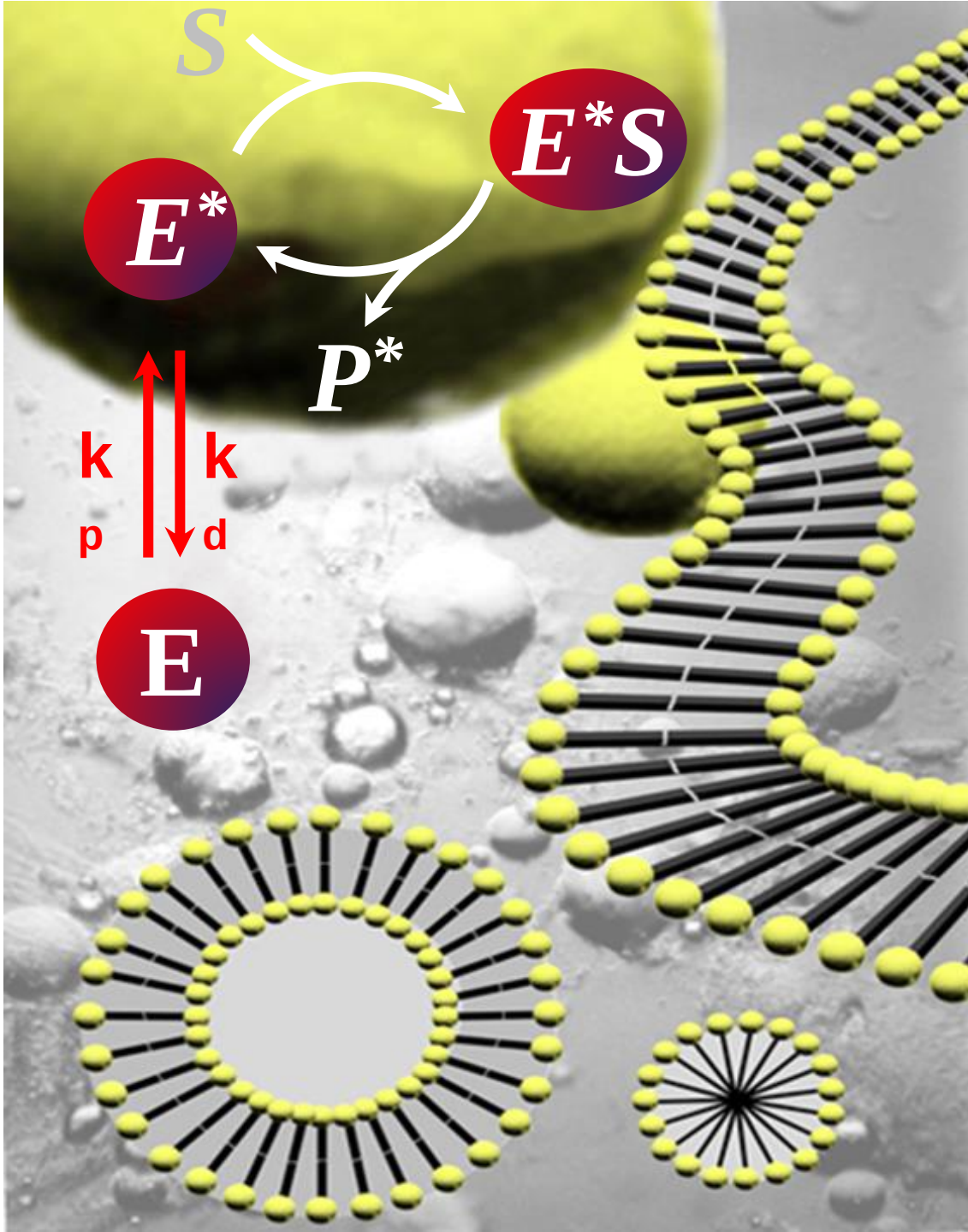
Lipase-Catalyzed Reactions



Heterogeneous Catalysis by Interfacial Enzymes

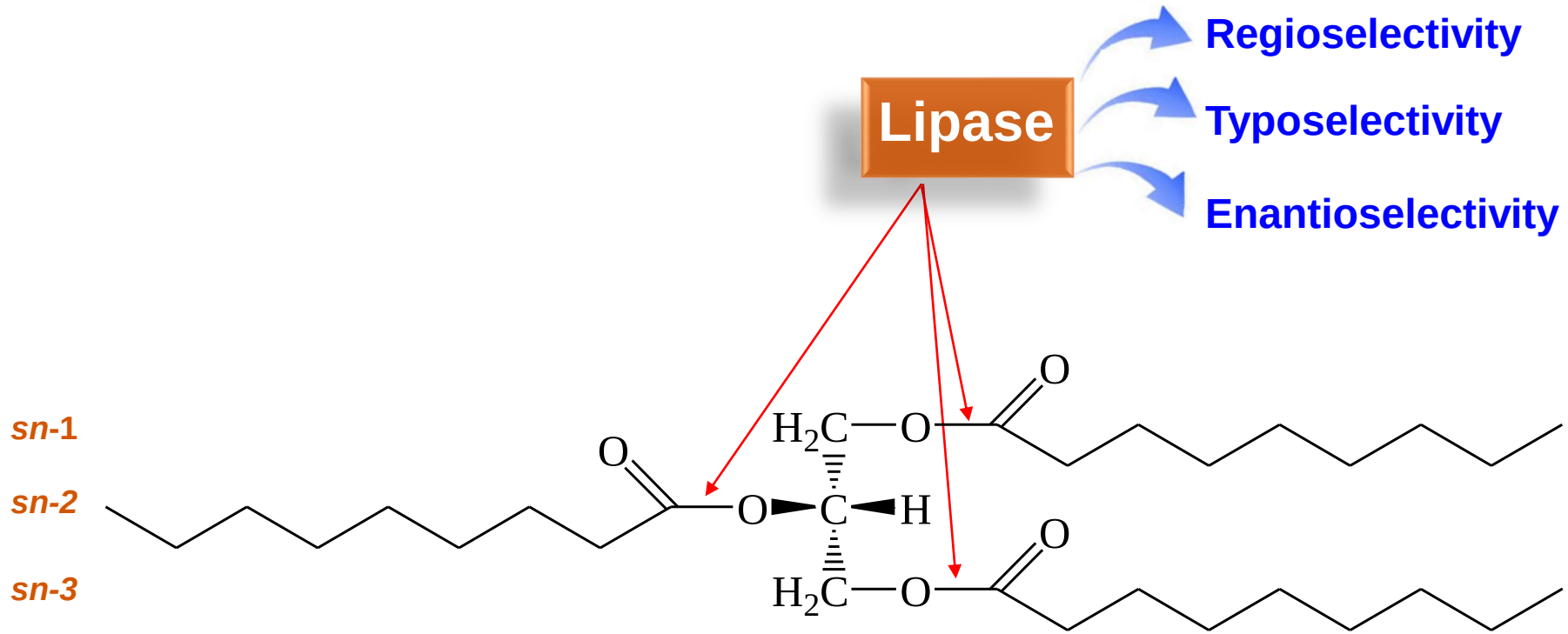
- Adsorption Step
- Catalytic Step

Substrate specificity of enzymes acting at interfaces can result from these two steps



Lipases

- Lipolytic Carboxylic Ester Hydrolases



Enzymatic lipophilization of HydroxyTyrosol

"Enzymatic lipophilization" is the use of biocatalysts (generally lipases) to allow lipophilic molecules of the fatty acid or fatty alcohol type to be grafted onto hydrophilic biomolecules.

This lipophilization process can be applied to different types of molecules and macromolecules such as amino acids, peptides, proteins or even sugars, phenolic acids or polyphenols.

Lipophilization is a solution for considerably reducing the polarity of the phenolic monomers by grafting aliphatic chains of different lengths and for adjusting the oil / water distribution in the multiphasic food matrices.

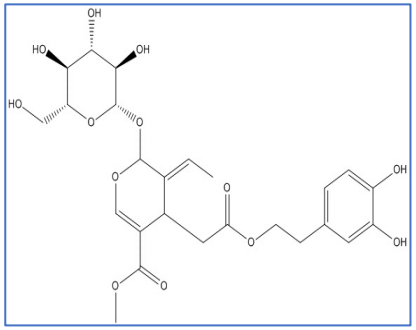
Sugar esters are currently the most widely produced lipophilic molecules.

They are obtained by grafting **fatty acids** onto different types of **sugars** and find their applications as **emulsifiers** mainly in the food and cosmetics industries.

In addition, **antibacterial**, **anti-tumor,** and **insecticidal** properties have been attributed to them (Lang et al., 2000).

- Molecular Formula: C25H32O13
- Molecular Weight: 540.5138g/mol
- Plant Source: Olive Tree
- Part Used: Leaf

Oleuropein



Oleuropein, a bioactive compound found in all parts of olive tree, especially in leaves and branches, presents numerous health promoting properties that increase research and market interest the last few years.

In addition, oleuropein degradation products, such as hydroxytyrosol, elenolic acid, and the aglycones also exhibit biological activities with different properties compared to the starting compound.

Extraction and identification of the major compound of the leaves of the olive tree

- Oleuropein is the main and dominant phenol in olive leaves

- Several solvent mixtures:

* MeOH / H_2O (8:2 v/v);
* EtOH / H_2O (5:5 v/v) ;
* MeOH / H_2O (5:5 v/v) ;

Water ; ethanol and methanol.

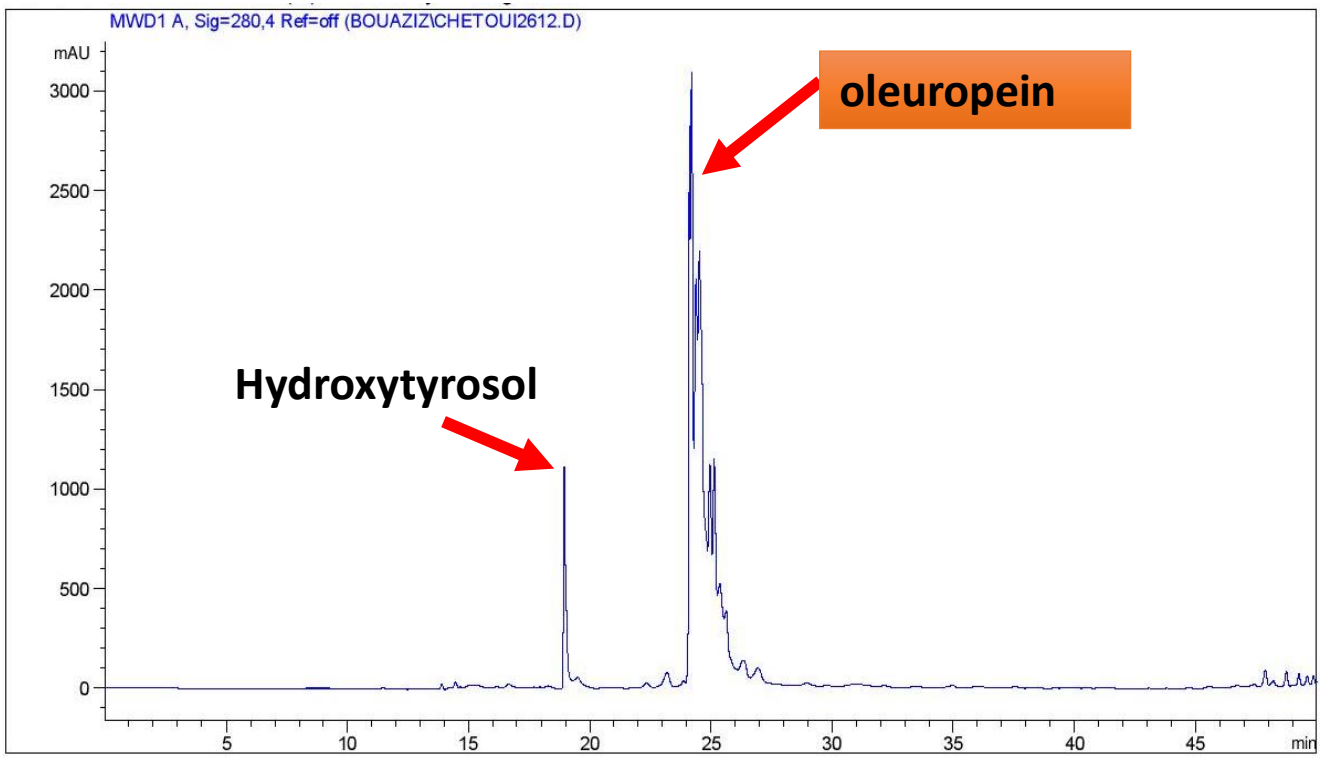
Extraction Solvent	CH_3OH	CH_3OH/H_2O (8:2)	CH_3OH/H_2O (5:5)	C_2H_5OH	C_2H_5OH/ H_2O (5 :5)	H_2O
Oleuropein (%. w/w)	8.7	14.2	10.2	4.7	7.5	6.4

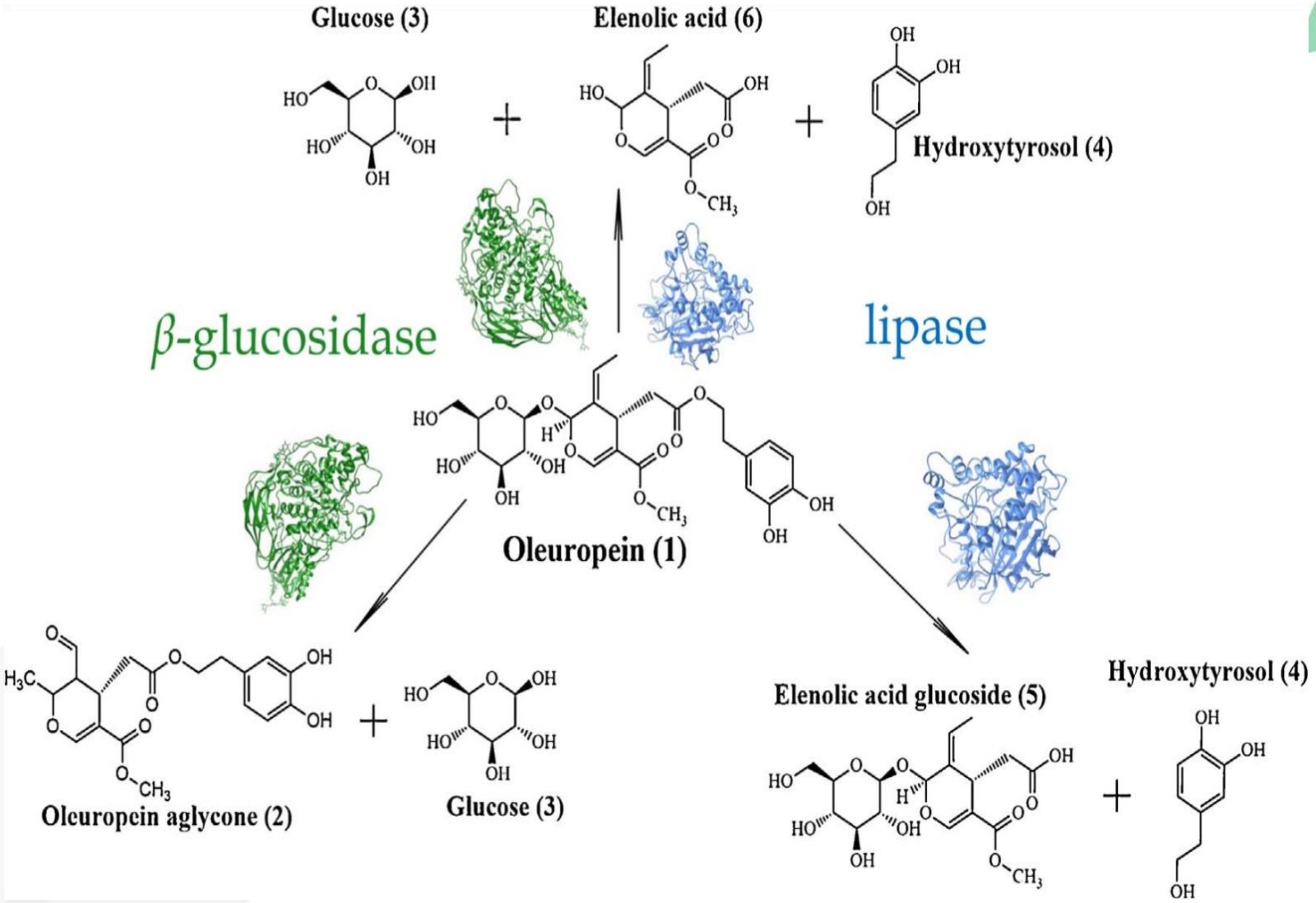
Extraction of oleuropein by ethyl acetate

Preparation of plant material

The samples were lyophilized and then extracted by maceration using **ethyl acetate**. It has been demonstrated that this solvent is better than others to extract phenolics.

Solvent has been replaced for 3 times and obtained extracts have been dried using a rotary evaporator.

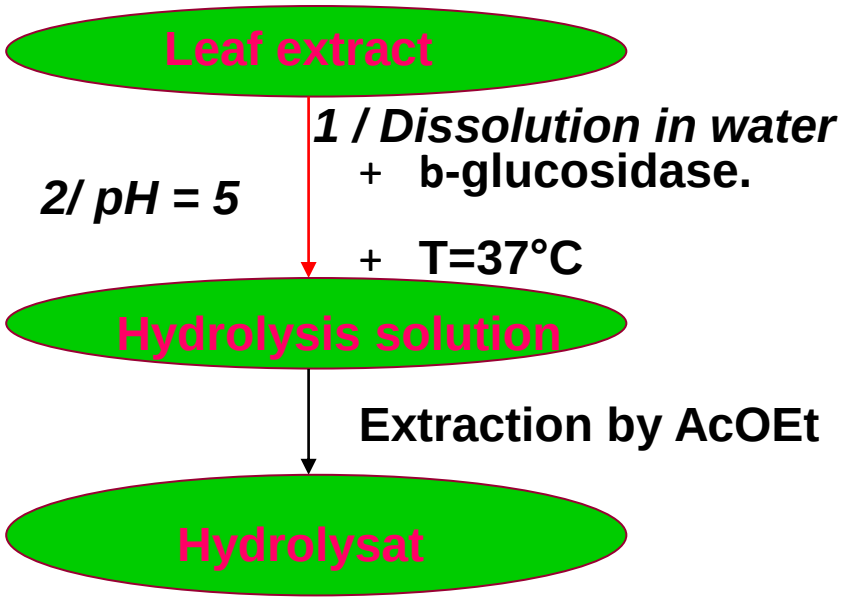




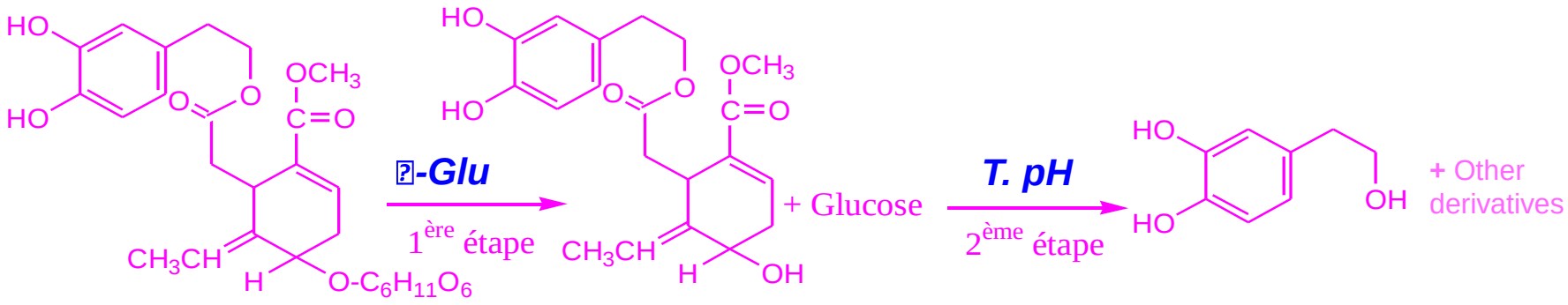
(Nikolaivits,E et al , 2017).

Enzymatic Production of hydroxytyrosol from oleuropein

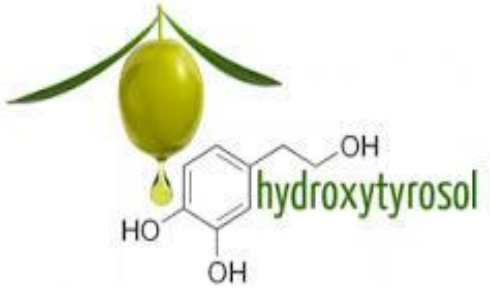
Enzymatic Process



- **Enzymatic hydrolysis reaction is done in two steps:**



Oleuropéine — Oleuropéine Aglycone — Hydroxytyrosol

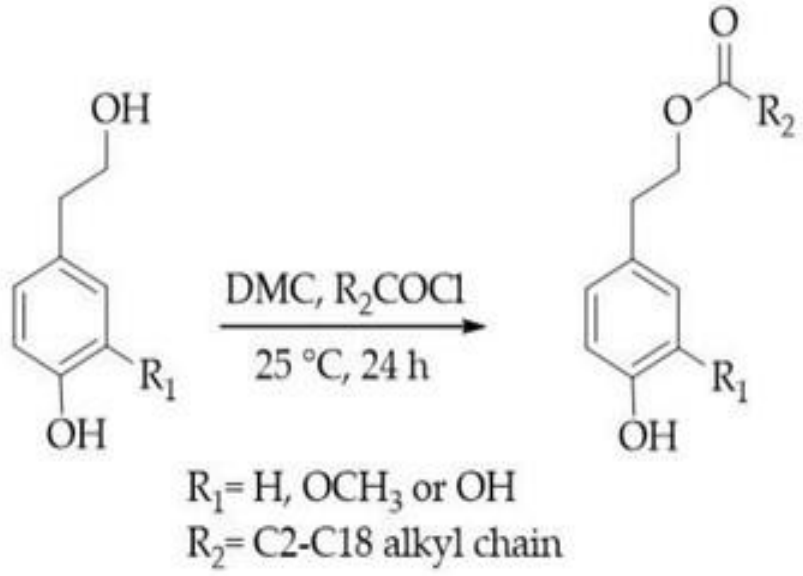


Hydroxytyrosol

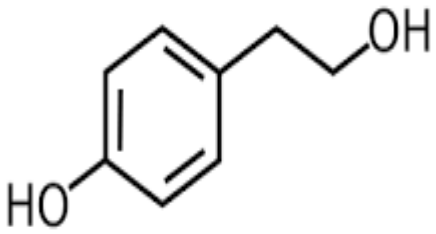
- Hydroxytyrosol is the major compound in the crude ethyl acetate extract of in olive leaves .

- 100 mg ⟶ 700

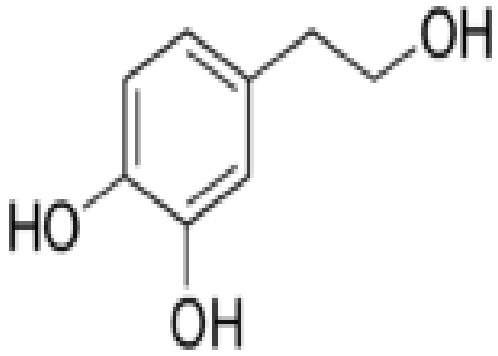
- Due to its interesting biological activities, hydroxytyrosol is considered as a molecule with high added value.



Tyrosol is an antioxidant phenolic compound, found especially in olive leaves



Tyrosol



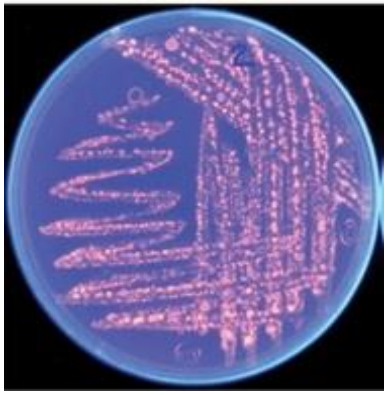
Hydroxytyrosol

Low-molecular weight phenols found in olive leaves.

Screening of lipase-producing

Purification of *Serratia* sp. W3 Lipase

Biochemical characterization of *Serratia* sp. W3

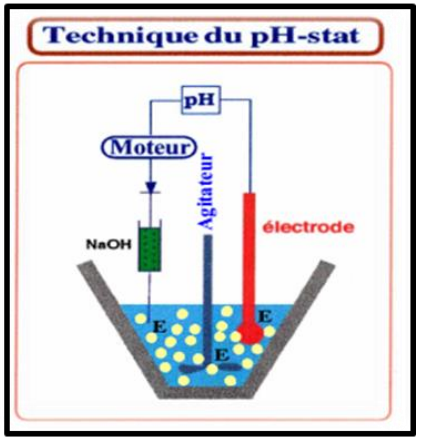


LSm

Specific activity : 625 U/mg (TC4) et 300 U/mg HO emulsion

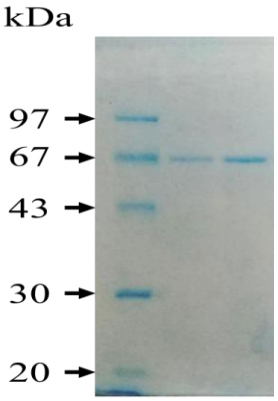
Yield : 10%

Purification Factor : 157



***Serratia* sp. w3**

N. d'accession MH762127.



Serratia sp. W3 presented interesting biochemical criteria suggesting that this enzyme could be a suitable candidate for biological transformations in the food industries.

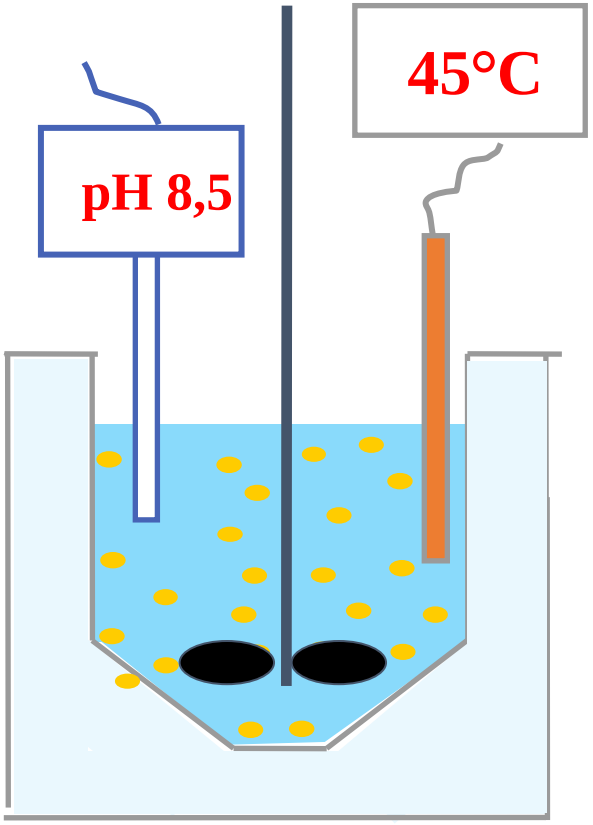
Enzymatic synthesis of new bioactive compounds

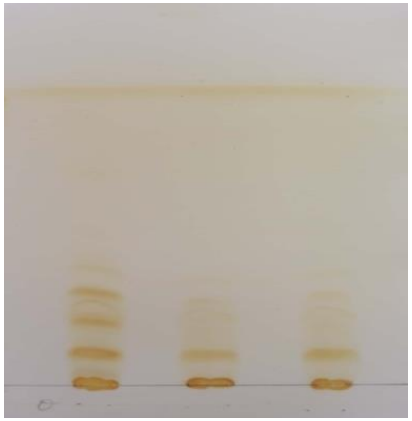
Esterification conditions were optimized for the synthesis of hydroxytyrosol-Fatty acid using immobilized lipase from *Serratia* sp W3 and Candida antarctica B (Novozym 435) and were applied to the synthesis of all conjugates.

An immobilized lipase from *Serratia* sp. W3

Physical adsorption: Efficient immobilization

- Oleuropein: 0.06g
- Ethyl acetate: 5ml
- The quantity of enzyme 0.32 g, 40 IU
- Incubation: 24 h
- 150 rpm



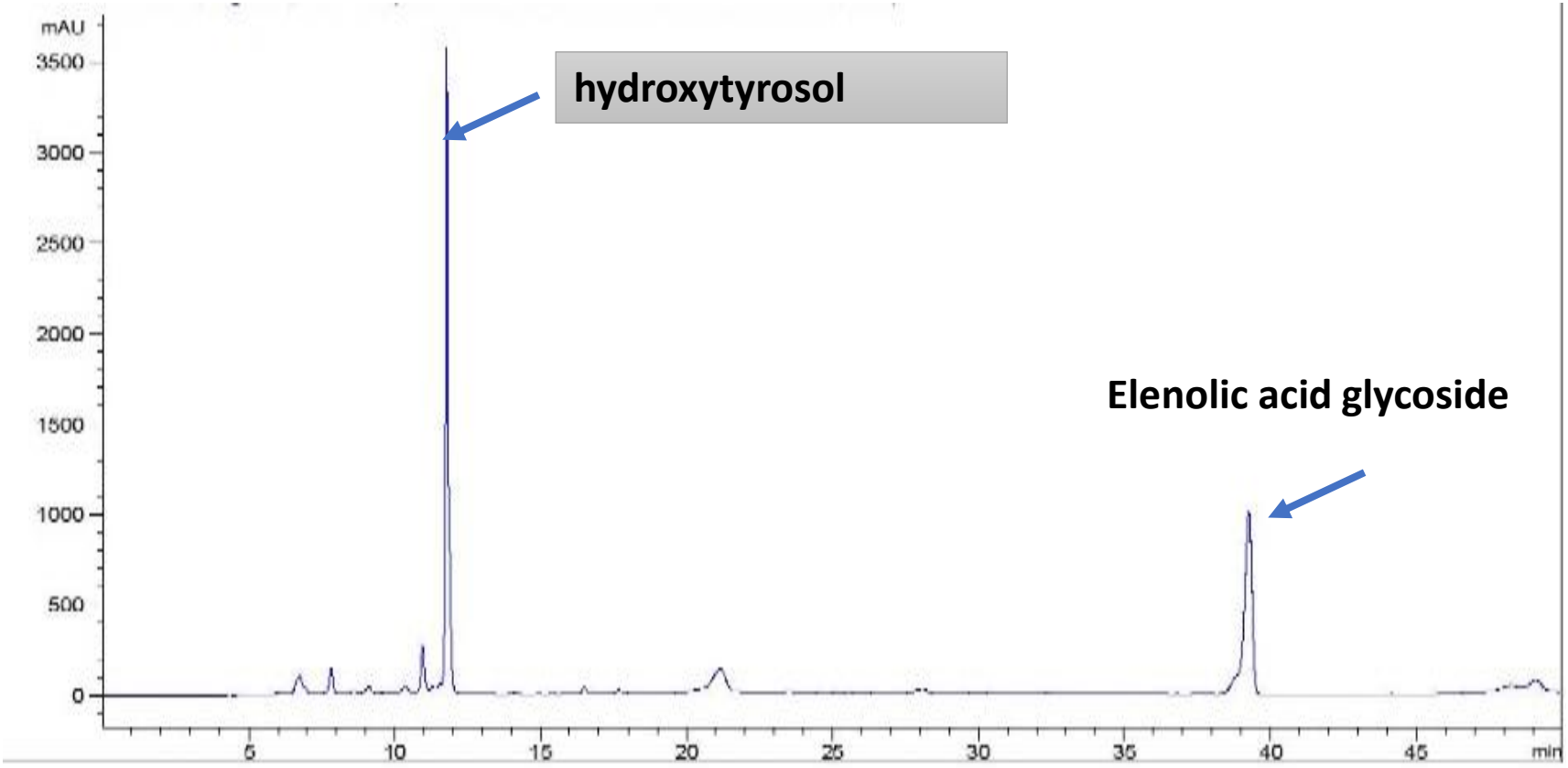


1 2 3

1: Oleuropein after extraction with ethyl acetate.

2: Product after reaction by *serratia* sp.w3 lipase.

Using an immobilized lipase from Serratia sp. W3

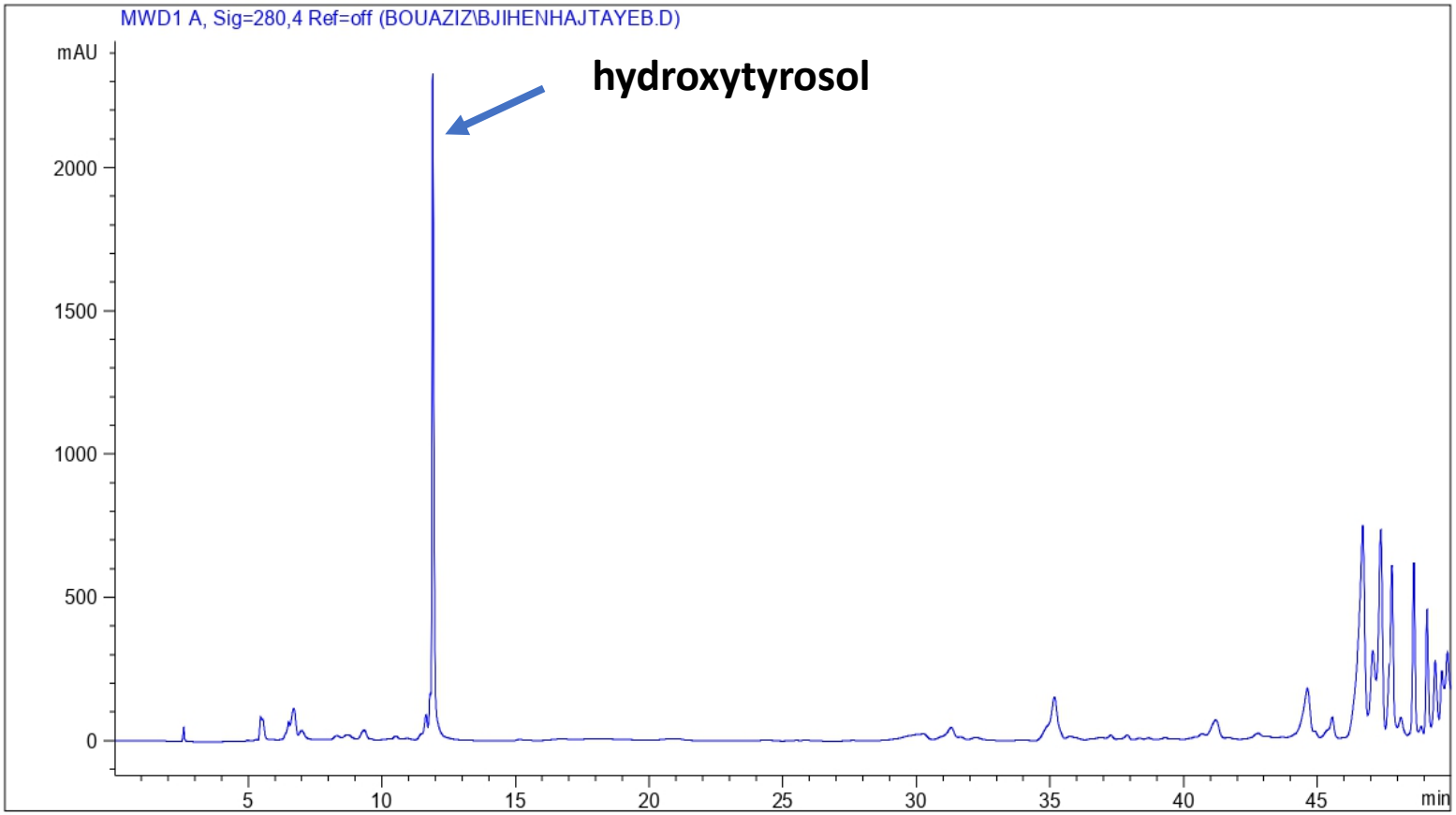


HPLC chromatogram

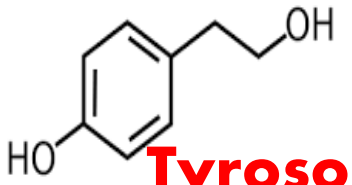
Using an immobilized lipase from Serratia sp. W3

Hydrolysis

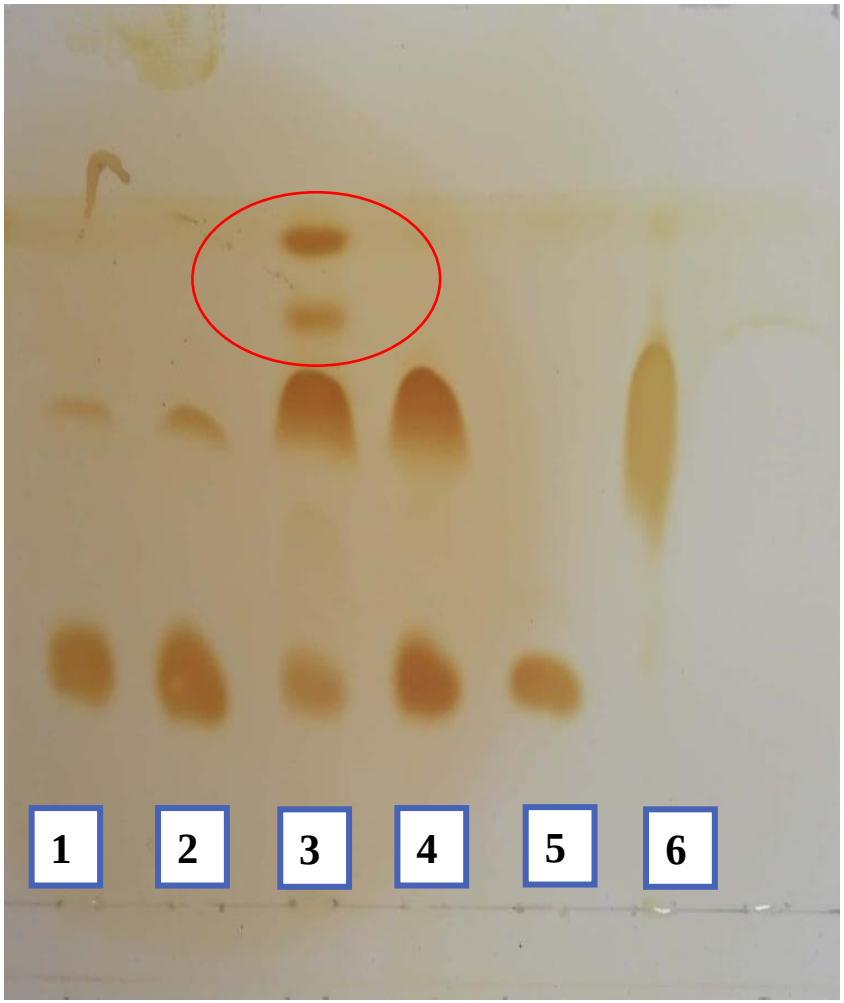
Synt[illegible]s(Esterification)



Synthesis reaction-Tyrosol-Fatty acid



Tyrosol



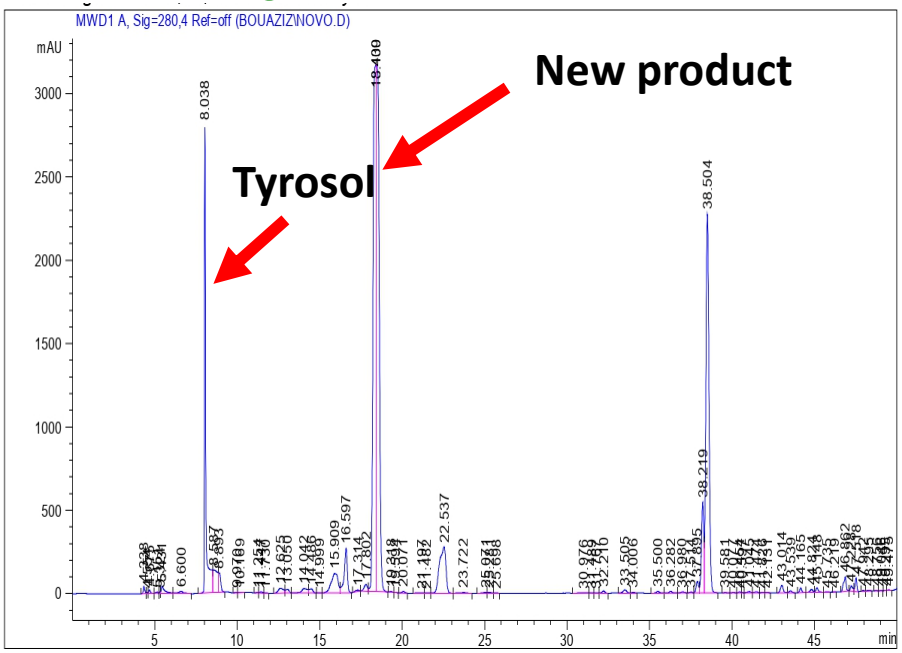
lipase *Serratia* sp.w3

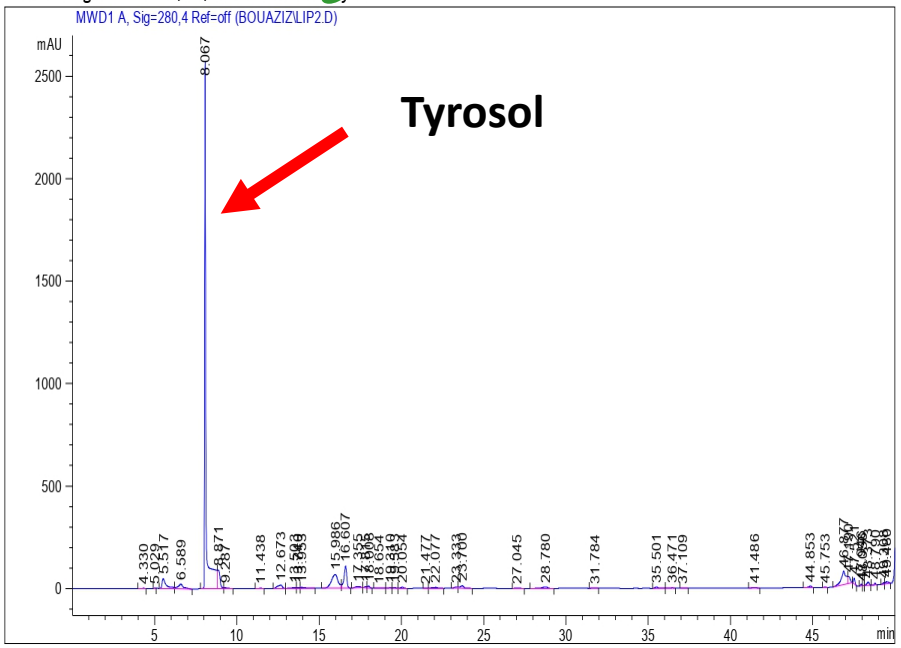
lipase "Novozyme"

1: Synthesis reaction in the presence of enzyme with a 2:1 molar ratio (tyrosol/acetic acid) after 24 hours of incubation.
2: Synthesis reaction in the absence of enzyme with a 2:1 molar ratio (tyrosol/acetic acid) after 24 hours of incubation.
3: Synthesis reaction in the presence of **novozyme 435** with a 2:1 molar ratio (tyrosol/oleic acid) after 24 hours of incubation.
4: Synthesis reaction in the absence of enzyme with a 2:1 molar ratio (tyrosol/oleic acid) after 24 hours of incubation.

5: tyrosol
6: oleic acid

Synthesis reaction-Tyrosol-Fatty acid

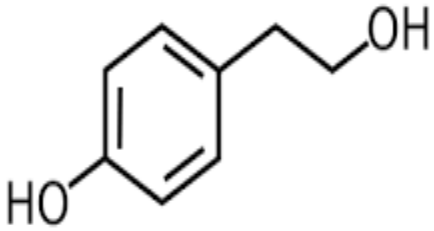




lipase "Novozyme"

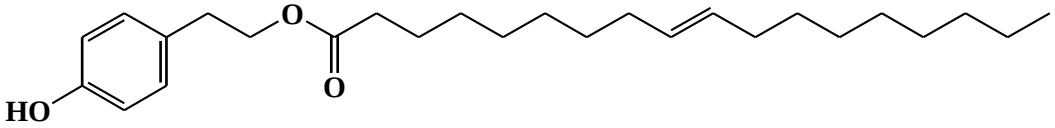
lipase from *Serratia* sp.w3

Tyrosol



20 mg ---- 300 €

Tyrosol oleate ($TyC_{18:1}$)



Structures of Tyrosol Derivatives

Tyrosol (Ty)

Tyrosol acetate (TyC_2)

Tyrosol propionate (TyC_3)

Tyrosol Caprylate (TyC_8)

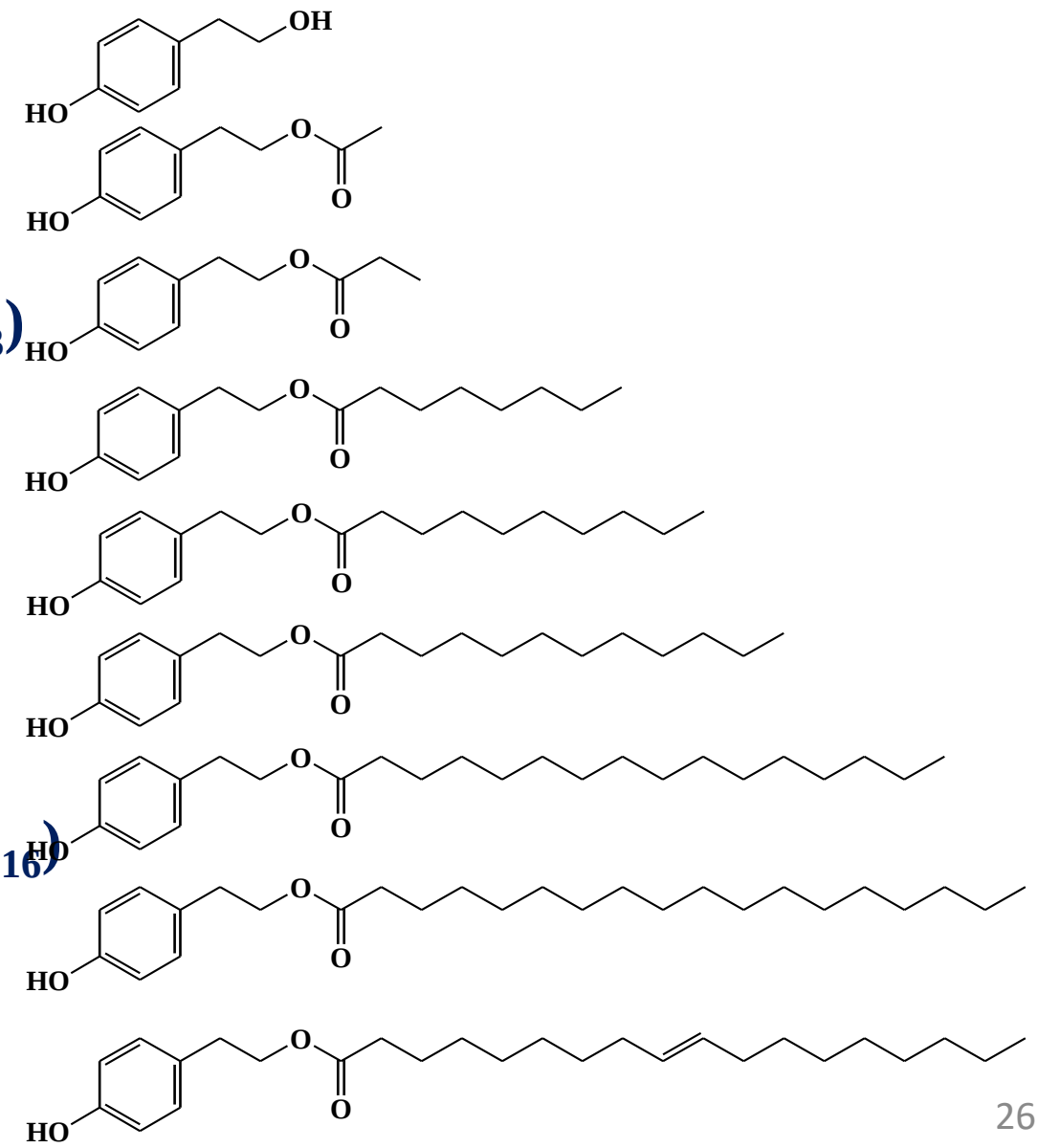
Tyrosol caprate (TyC_{10})

Tyrosol Laurate (TyC_{12})

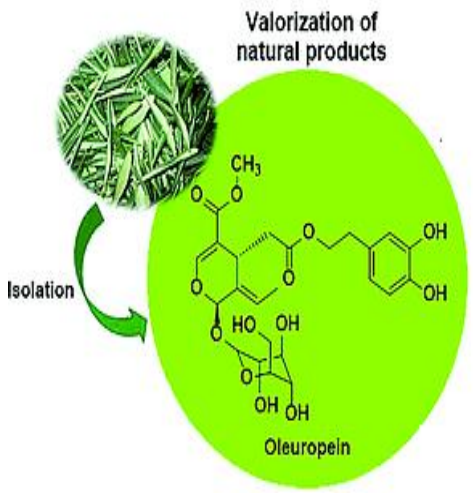
Palmitate of tyrosol (TyC_{16})

Tyrosol Stearate (TyC_{18})

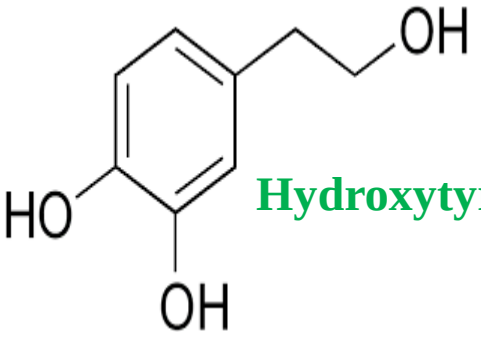
Tyrosol oleate ($TyC_{18:1}$)



Combinations of two enzymatic reactions



lipase from *Serratia* sp.w3



Hydroxytyrosol

Enzymatic Hydrolysis

enzymatic reaction I

oleic acid + **lipase "Novozyme"**

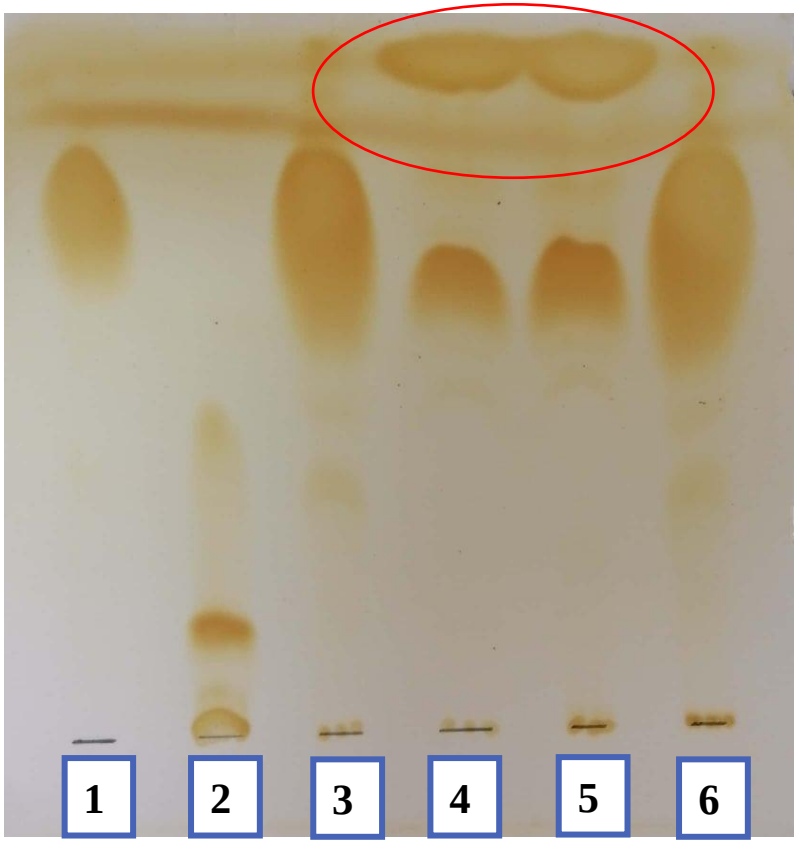
HYPOTHESIS

Hydroxytyrosol derivative

enzymatic reaction II

Hydroxytyrosol oleate (H-Ty$C_{18:1}$)

Synthesis reaction-Hydroxytyrosol-Fatty acid



1: oleic acid

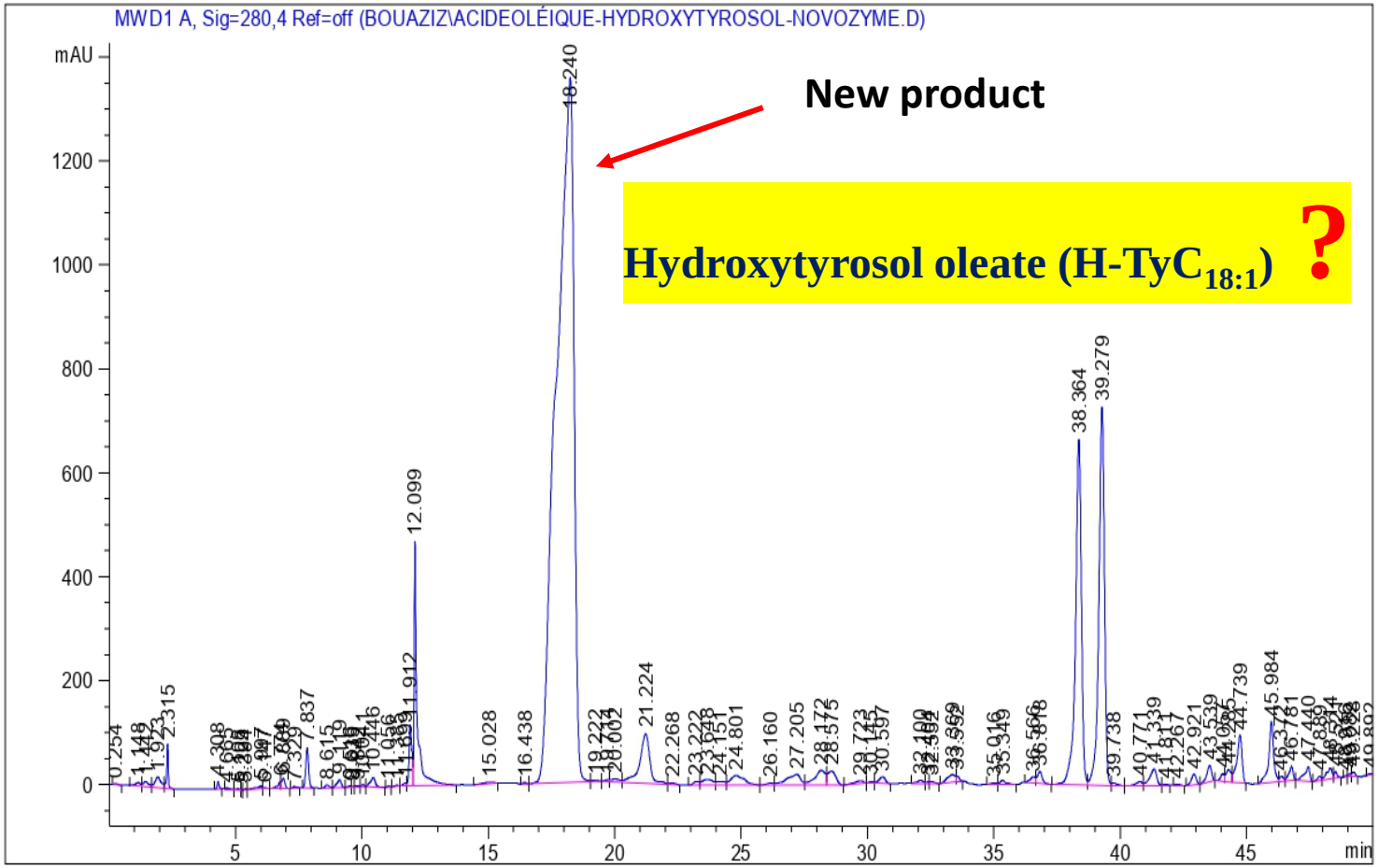
2: Oleuropein

3: lipase from *Serratia* sp.w3 with a 2:1 molar ratio (Hydroxytyrosol/Oleic acid) after 24 hours of incubation.
4: Novozyme 435 with a 2:1 molar ratio (Hydroxytyrosol/oleic acid) after 24 hours of incubation.

4: Novozyme 435 with a 2:1 molar ratio (Oleuropein/oleic acid) after 24 hours of incubation.

3: Lipase from *Serratia* sp.w3 with a 2:1 molar ratio (Oleuropein/oleic acid) after 24 hours of incubation.

Combinations of two enzymatic reactions



Products derived enzymatically from oleuropein will be identified by LC/MS and NMR.

Products will be tested in emulsions/ Stabilization of emulsions/Effects of Antioxidant Hydrophobicity/ Oxidative Stability of the Emulsions/

FunTomP

- PRIMA-H2020 (2021-2025) « Functionalized Tomato Products» Valorising the health benefits of the Traditional Mediterranean food products « FunTomP ».

ORTA DOĞU TEKNİK ÜNİVERSİTESİ
MIDDLE EAST TECHNICAL UNIVERSITY

Pr. Mecit Oztop

16 Partners

8 countries

Tomato juice and sauce

1% oleuropein powder

1% Hydroxytyrosol

1% Hydroxytyrosol-Fatty acid

1 % Biosurfactant

Analysis of tomato-based products

Quality Evaluation

Physical parameters:
-°Brix
-pH
-Eletrical Conductivity (EC)

Chemical parameters:
-Total phenolic compounds (TPC)
-Antioxidant capacity and stability

Zied ZARAI,
University of Sfax
High Institute of Biotechnology, Sfax,
Food Technology Department

zied.zarai@isbs.usf.tn

Thank you for your attention