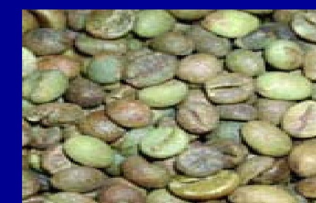
Enzymatic Lipophilization of phenolic compounds and evaluation of their antioxidant properties

Luis Javier López Giraldo



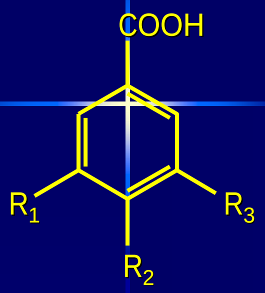
Prix de Thèse de l'Asociation Française pour l'Etude des Corps Gras

Microbial and enzymatic biotechnolgy of lipids and agropolymers team

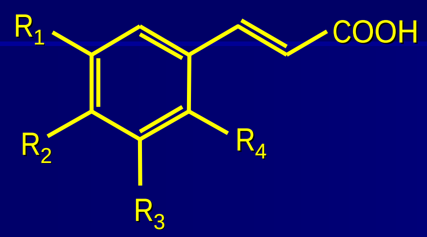
Directeur Pierre Villeneuve

Vegetable phenolic compounds

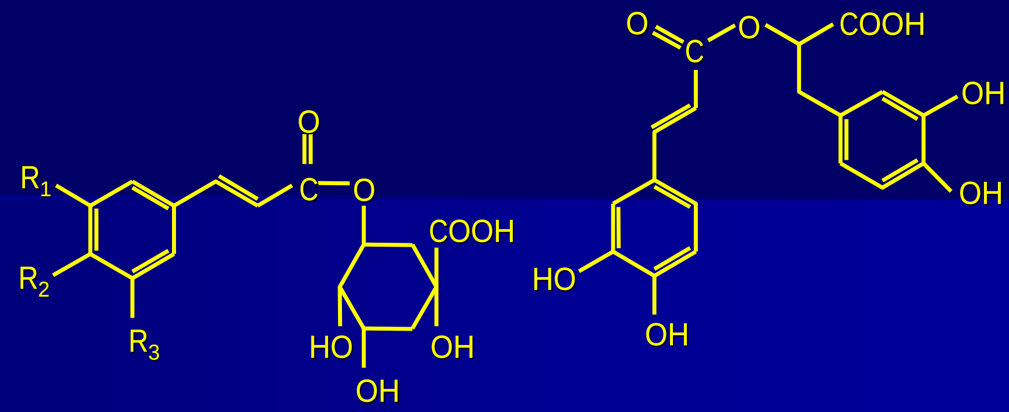
Phenolic Acids:



Hydroxybenzoic Acid

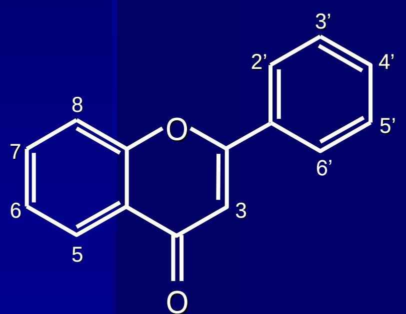


Hydroxycinnamic acid

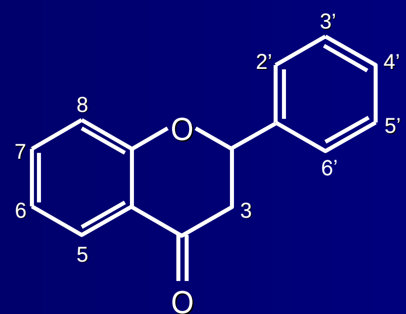


Hydroxycinnamic esters

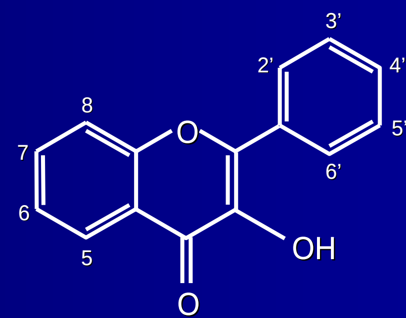
Flavonoids:



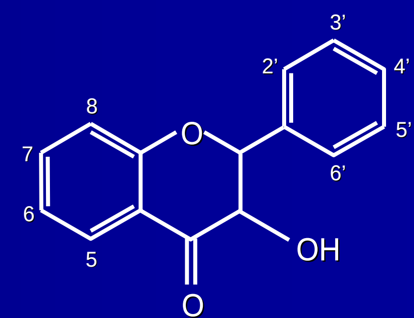
Flavons



Flavanons



Flavonols



Flavanols

Properties : Antioxidants, antimicrobial, anticancer

Structural features for antioxidant activity

1. Initiation

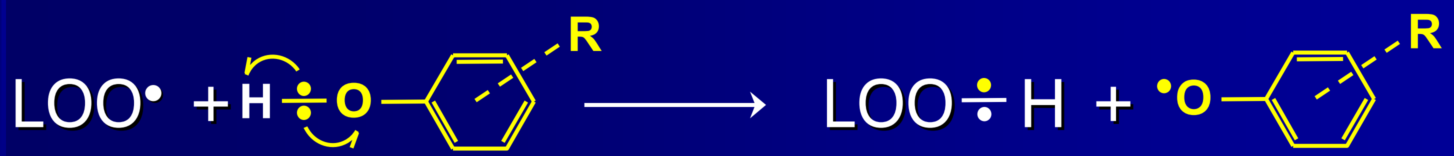
   $LH \rightarrow L^{\bullet} + H^{\bullet}$

2. Propagation

   $L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$

   $LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet}$

3. Termination



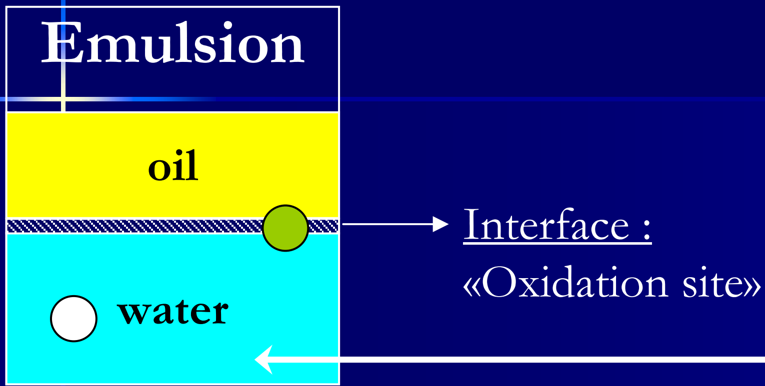
Second stabilization radical $LOO^{\bullet}$

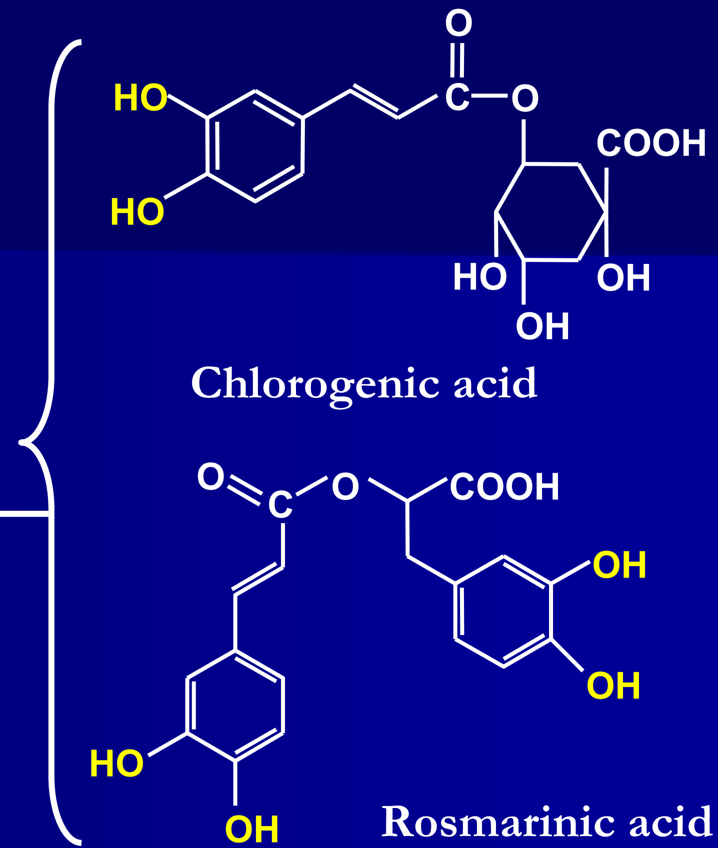
Intramolecular H bond

Dimerization

Regeneration

Interfacial phenomena and «Polar Paradox»



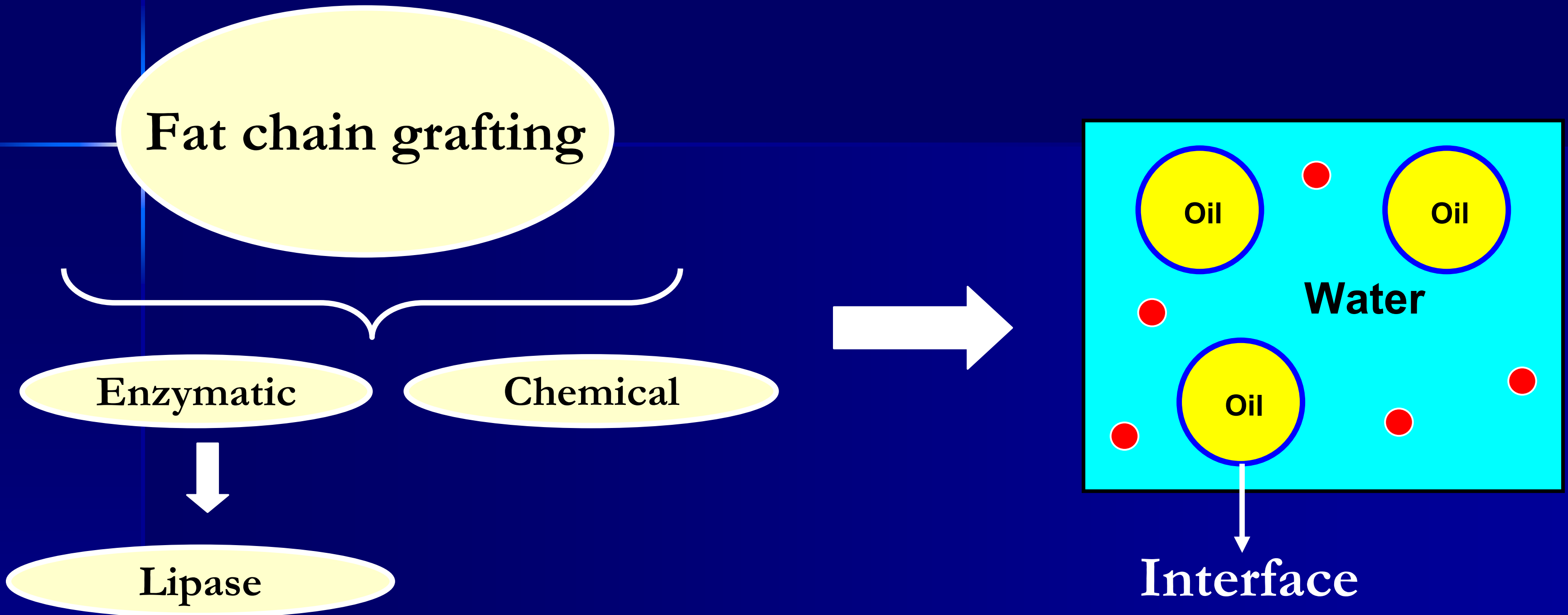


- Amphiphilic antioxidant
- Polar antioxidant

- **<u>Chlorogenic acid*</u>**
  - Green coffee extract: 100
  - Honeysuckle extract: 62
- **<u>Acide rosmarinique*</u>**
  - Rosemary extract : 40
  - Lemon balm extract: 2.2

(* g/kg dry matter)

Enzymatic lipophilization of phenolic compounds

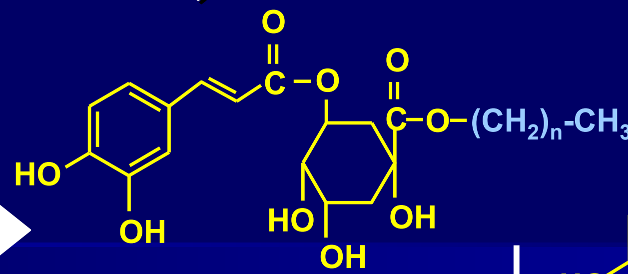


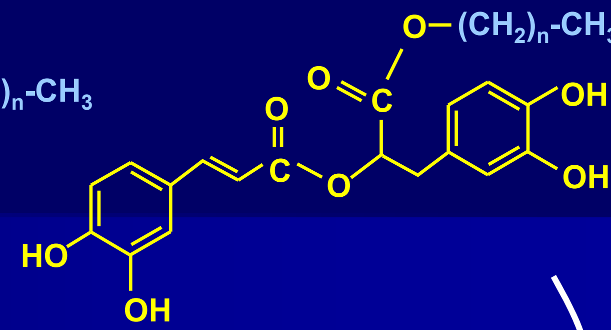
Advantages of Enzymatic synthesis:

- Milder reaction conditions
- Green Chemistry
- Reaction without solvent

Objectives

KEY PARAMETERS
LIPOPHILIZATION





- Substrates polarity
- a_w
- Catalyst %

Chain length impact on Antioxidant capacity

- H transfer mechanisms
- Becoming of phenoxyl radical

Homogenous DPPH method

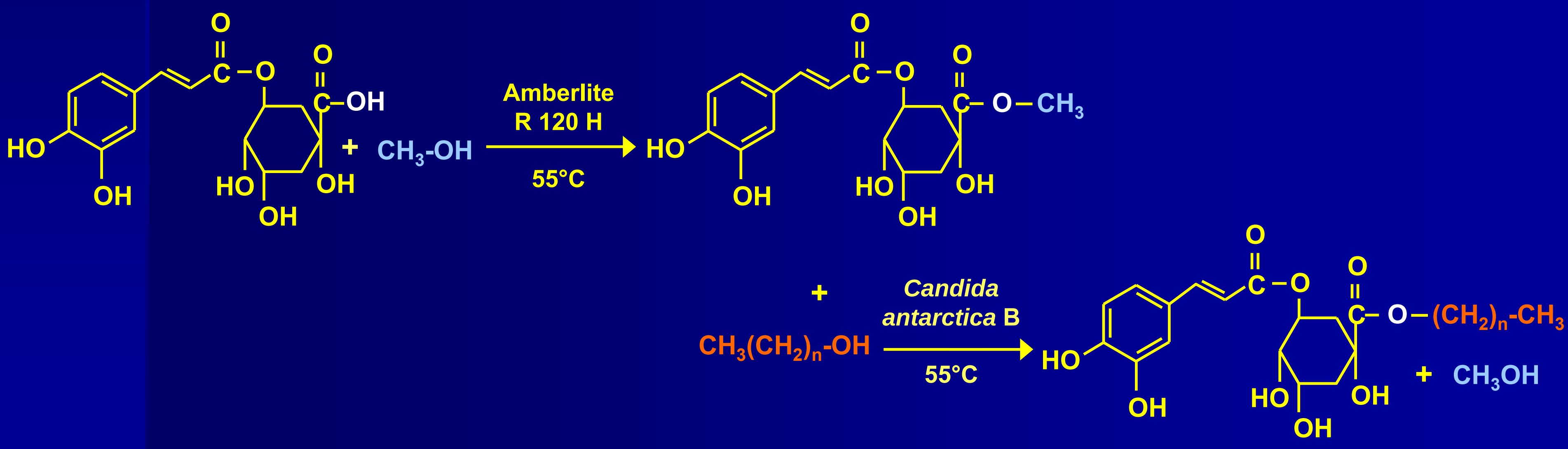
- Representative oxidation target
- Antioxidant distribution

Heterogeneous CAT method

Lipophilization strategies. Chlorogenic acid case

(a) Direct esterification

(b) Two steps strategy



(n = 3, 7, 11 et 15.)

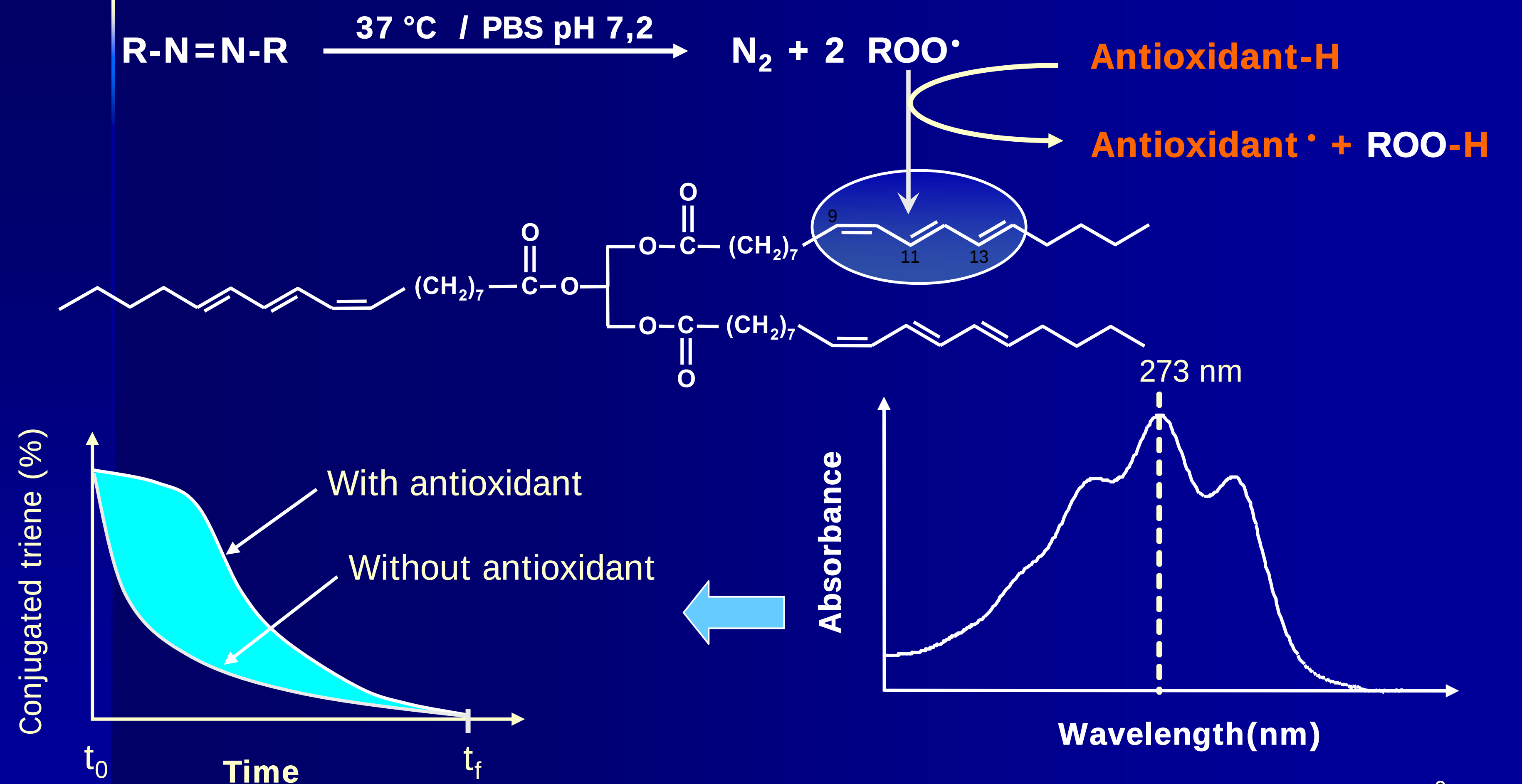
Summary of chlorogenic acid lipophilization

Conditions	Strategy	Molar yield (96 h)
■Reaction without solvent (alcohols C_4, C_8, C_{12}, C_{16}) ■55 °C ■5 % wt of lipase ■a_w= 0,05	Esterification	40-60 %
	Transesterification	63-93 %

Lopez-Giraldo LJ, *et al*. Enz. Microb.Tech. 2007, **41**, 721-726.

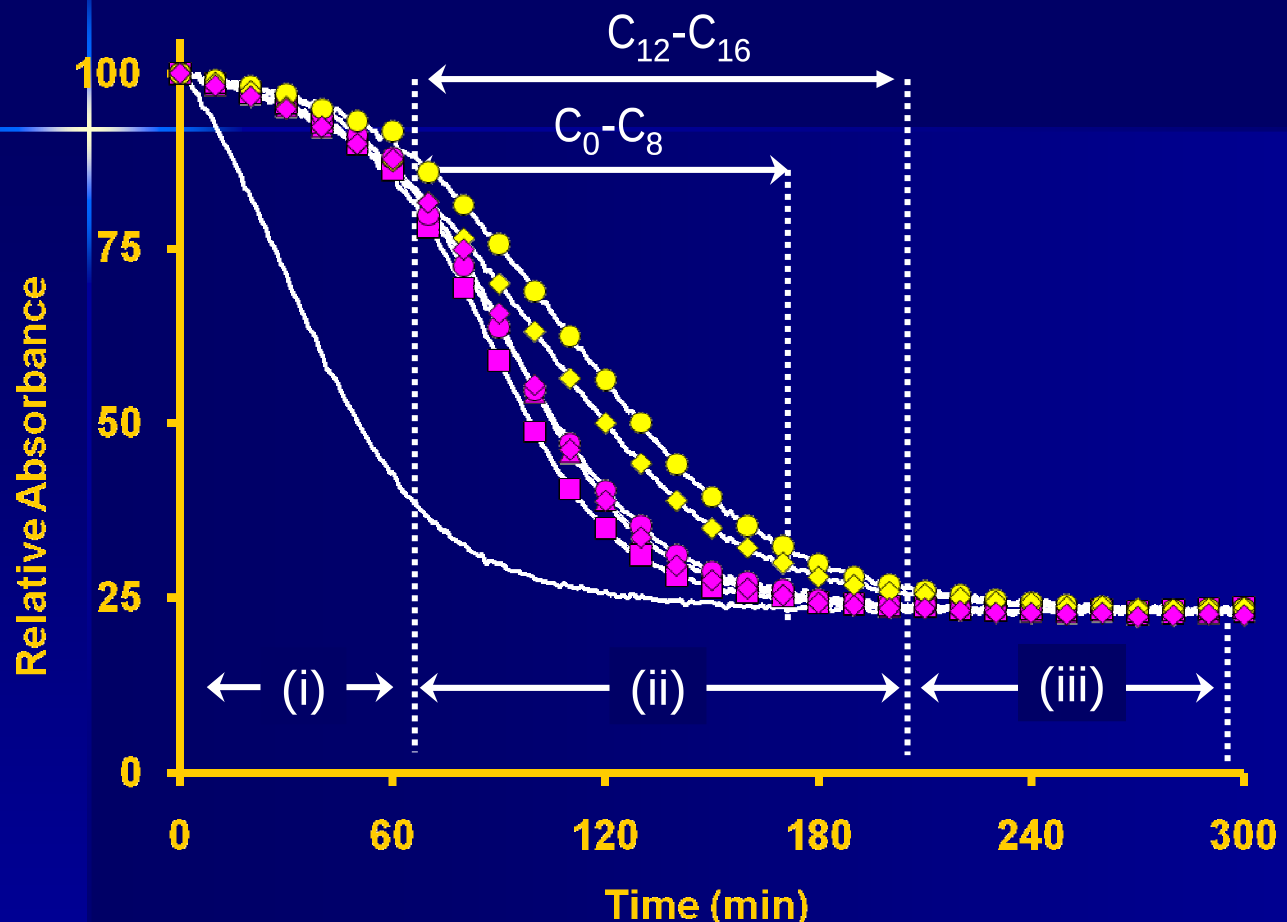
Antioxidant Capacity Evaluation by Conjugated autoxidizable triene (CAT)

- Principle



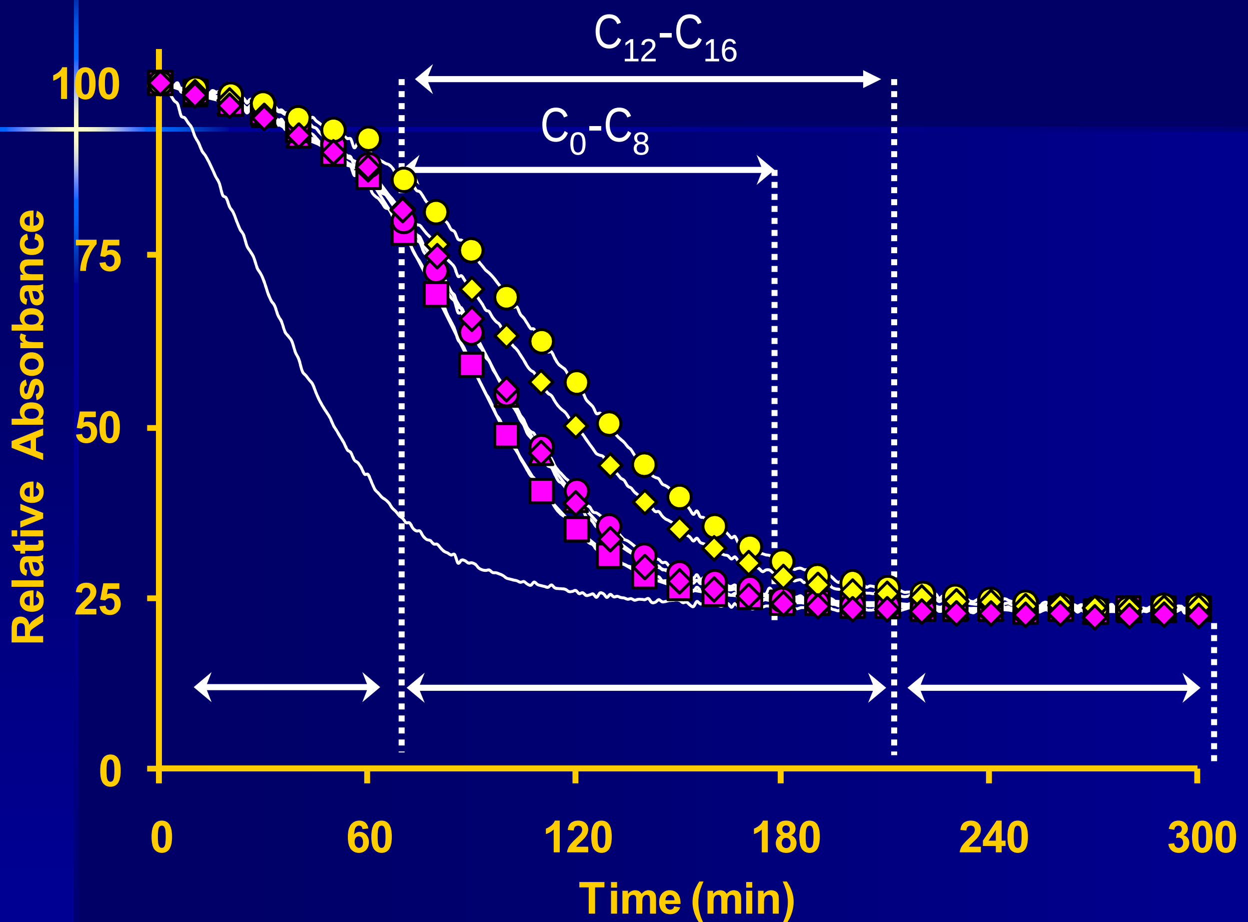
Laguerre *et al. Anal. Biochem. 2008*, ***380***, *282-290.*
Awards AOCS 2008

Kinetics of antioxidant capacity for chlorogenic acid and its esters



Kinetics of oxidation at 37°C without antioxidant(—) and with chlorogenic acid (♦), and its methyl (▲), butyl (■), octyl (●), dodecyl (●) and hexadecyl(♦) esters. Emulsioned system containing 115 µM of tung oil, 17 µM of Brij® 35 and 1 mM of AAPH.

Kinetics of antioxidant capacity for chlorogenic acid and its esters



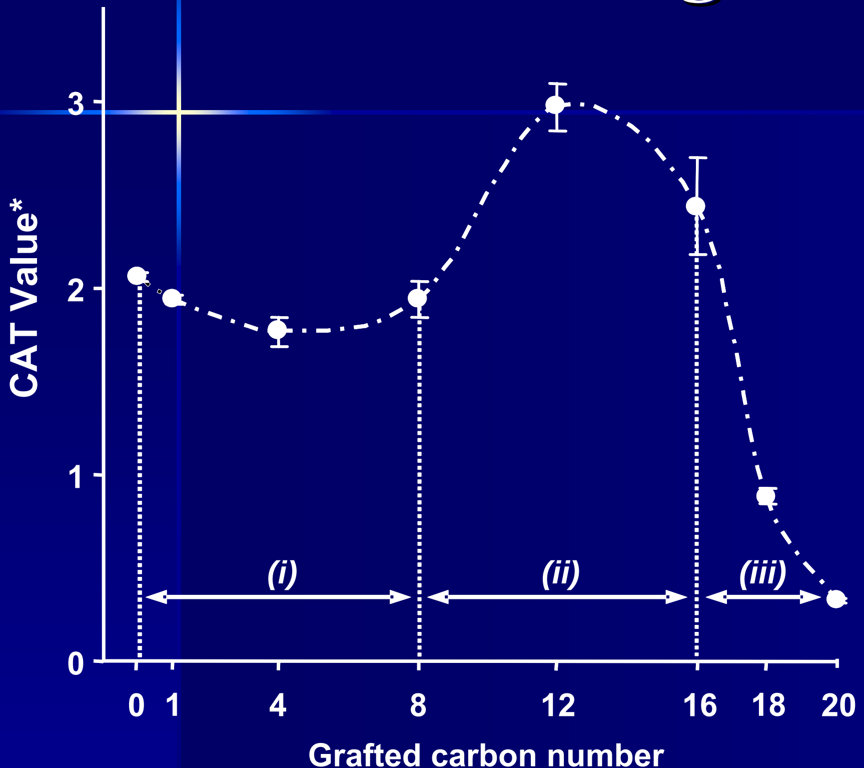
(i) Pseudo-inhibition phase

(ii) Decay phase

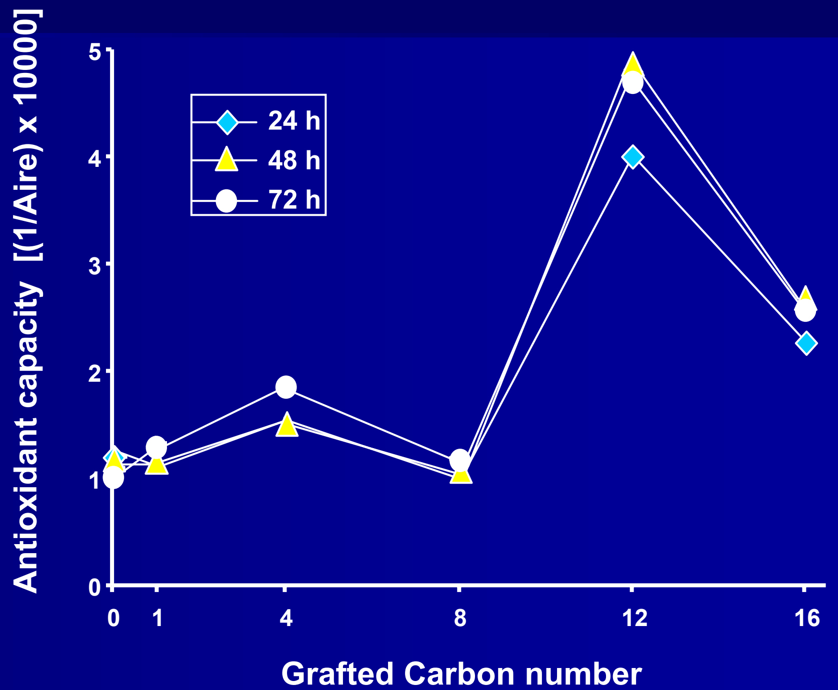
(iii) Stationary state

Kinetics of oxidation at 37°C without antioxidant(—) and with chlorogenic acid (♦), and its methyl (▲), butyl (■), octyl (●), dodecyl (●) and hexadecyl(♦) esters. Emulsioned system containing 115 µM of tung oil, 17 µM of Brij® 35 and 1 mM of AAPH.

Quantitative Evaluation of Antioxidant Capacity. Chlorogenic acid and its esters



Influence of chain length on the antioxidant capacity measured in emulsified system (test CAT)

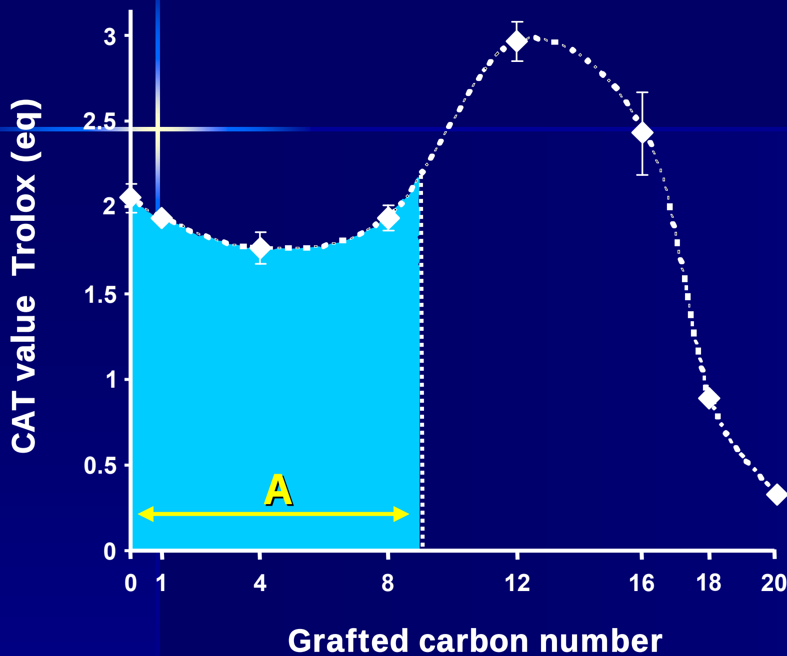


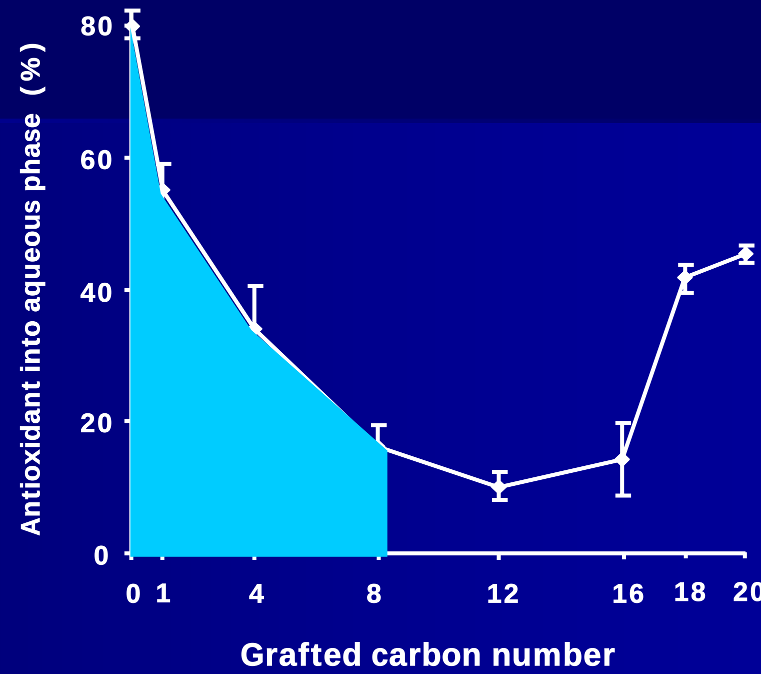
Influence of chain length on the antioxidant capacity measured in cellular system

- Polar Paradox *vs.* non linear results
- Results ~ in vitro cellular model

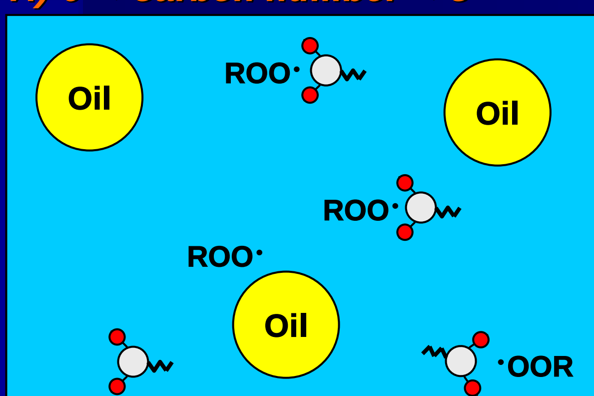
* Valeur CAT exprimée en mol d'eq. Trolox par mol de composé testé

Distribution and Antioxidant Capacity. Chlorogenic acid case

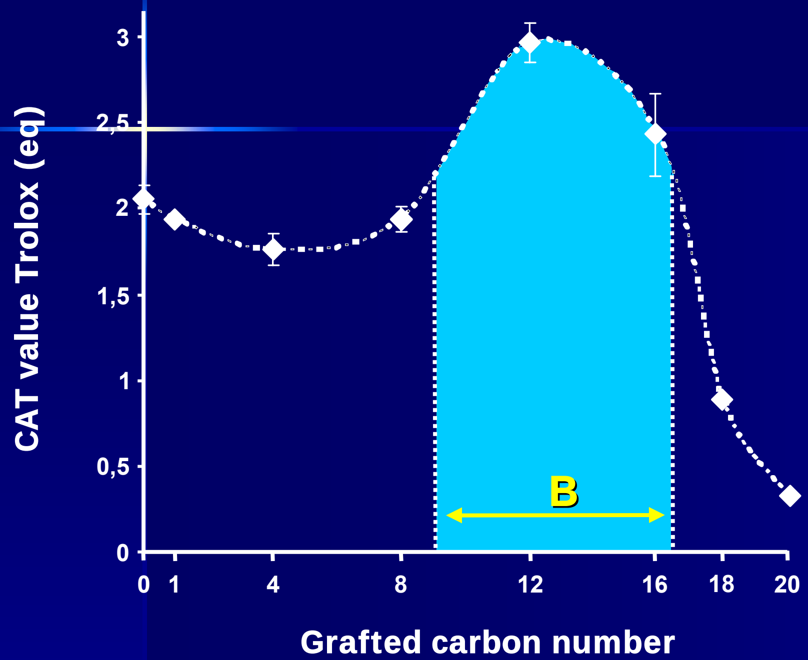


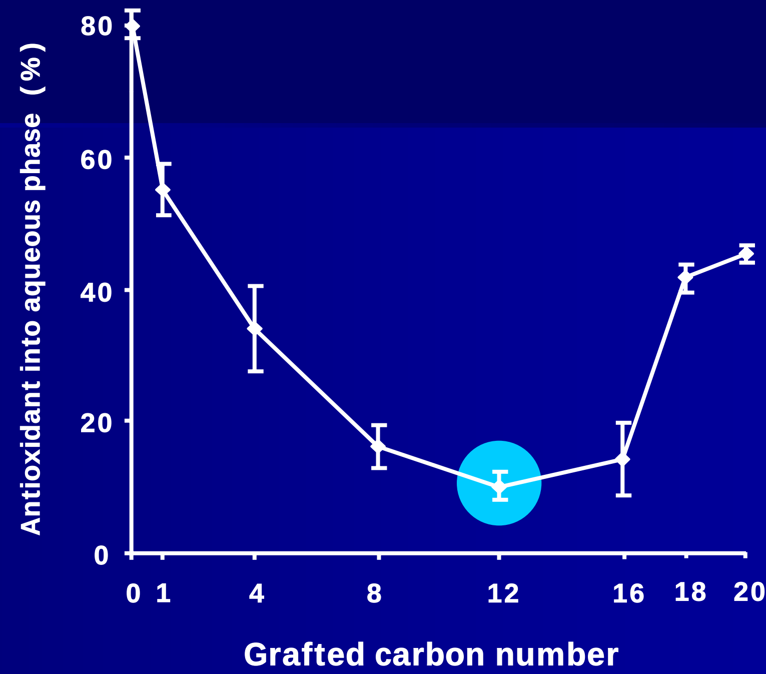


A) 0 < Carbon number < 8



Distribution and Antioxidant Capacity. Chlorogenic acid case





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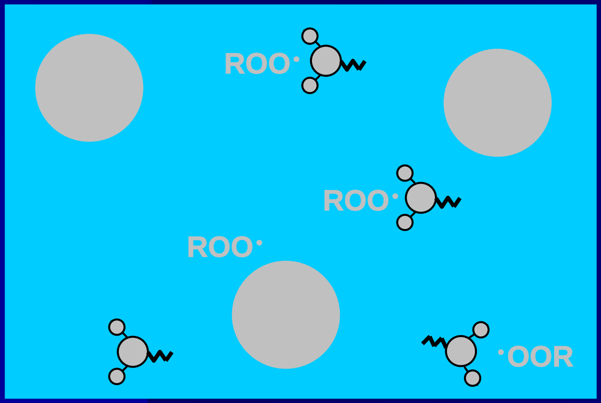
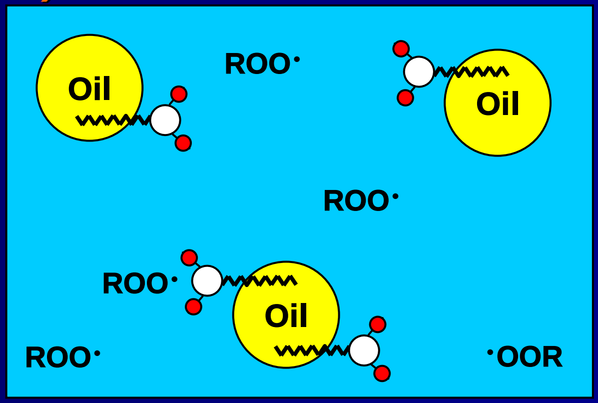
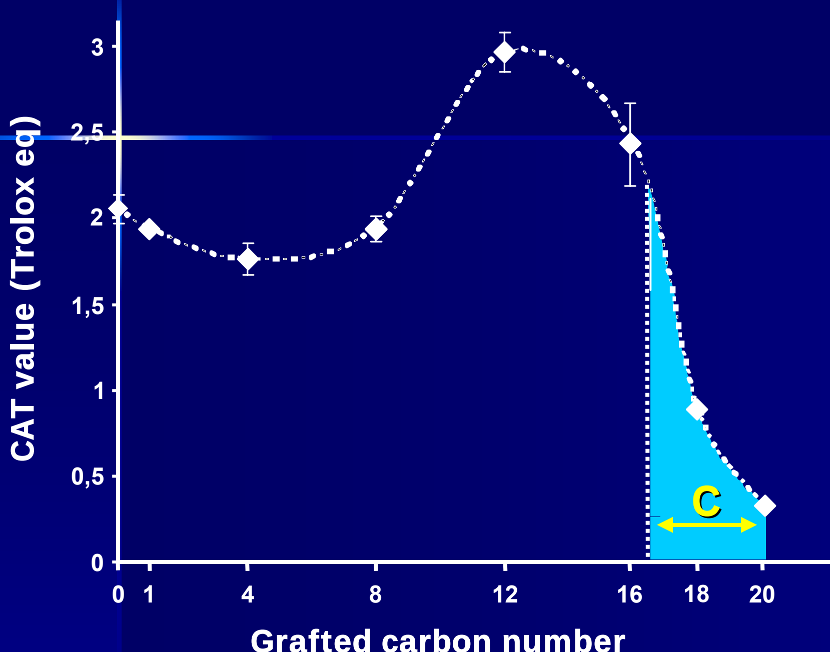
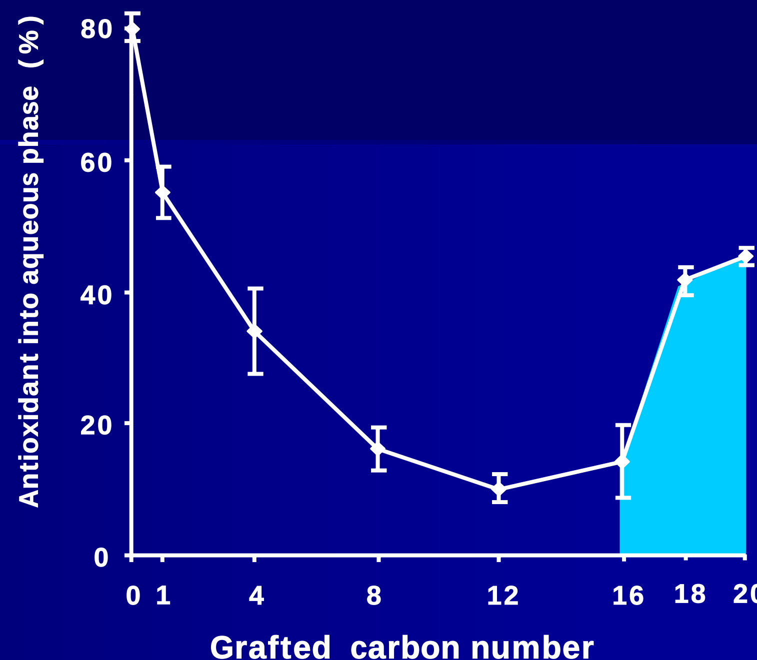


B) Carbon number = 12



Distribution and Antioxidant Capacity. Chlorogenic acid case





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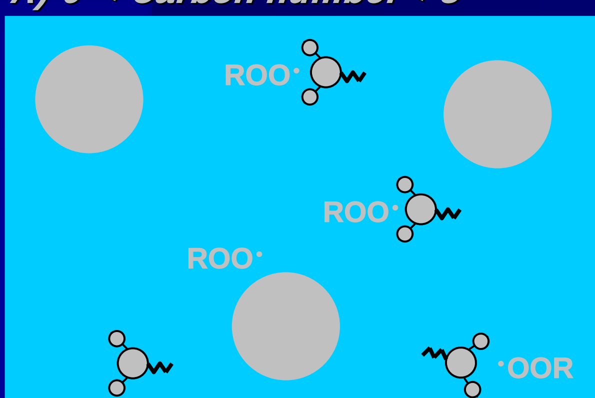

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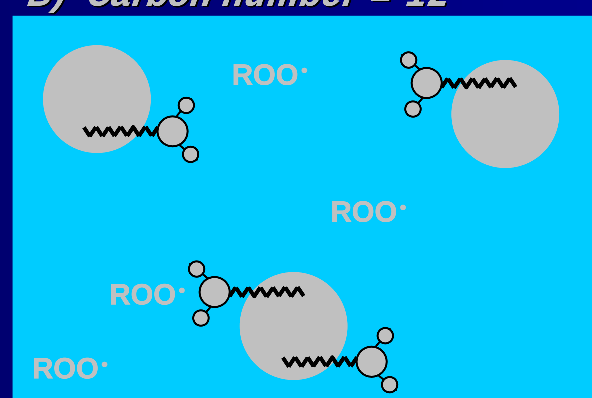
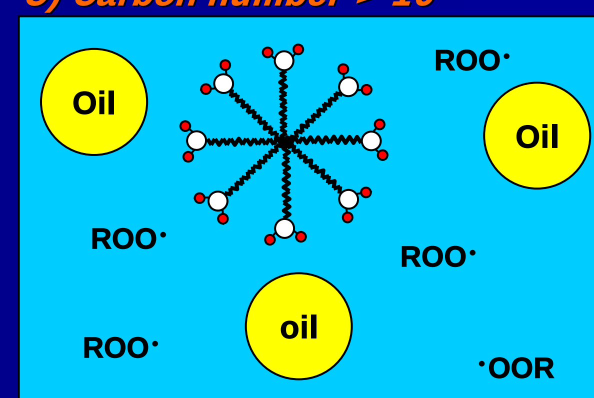
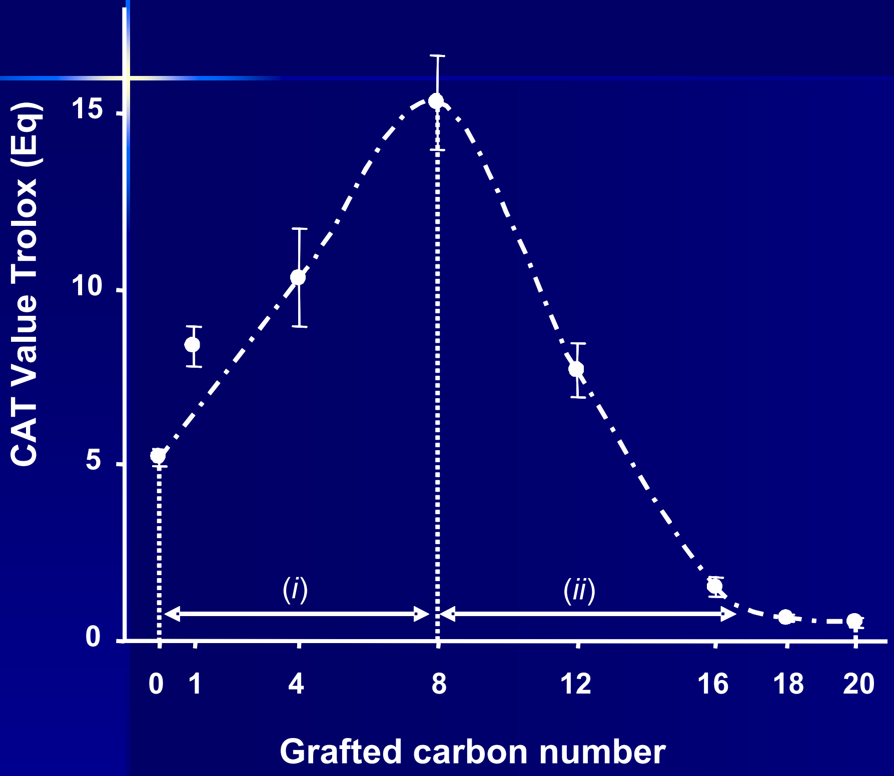


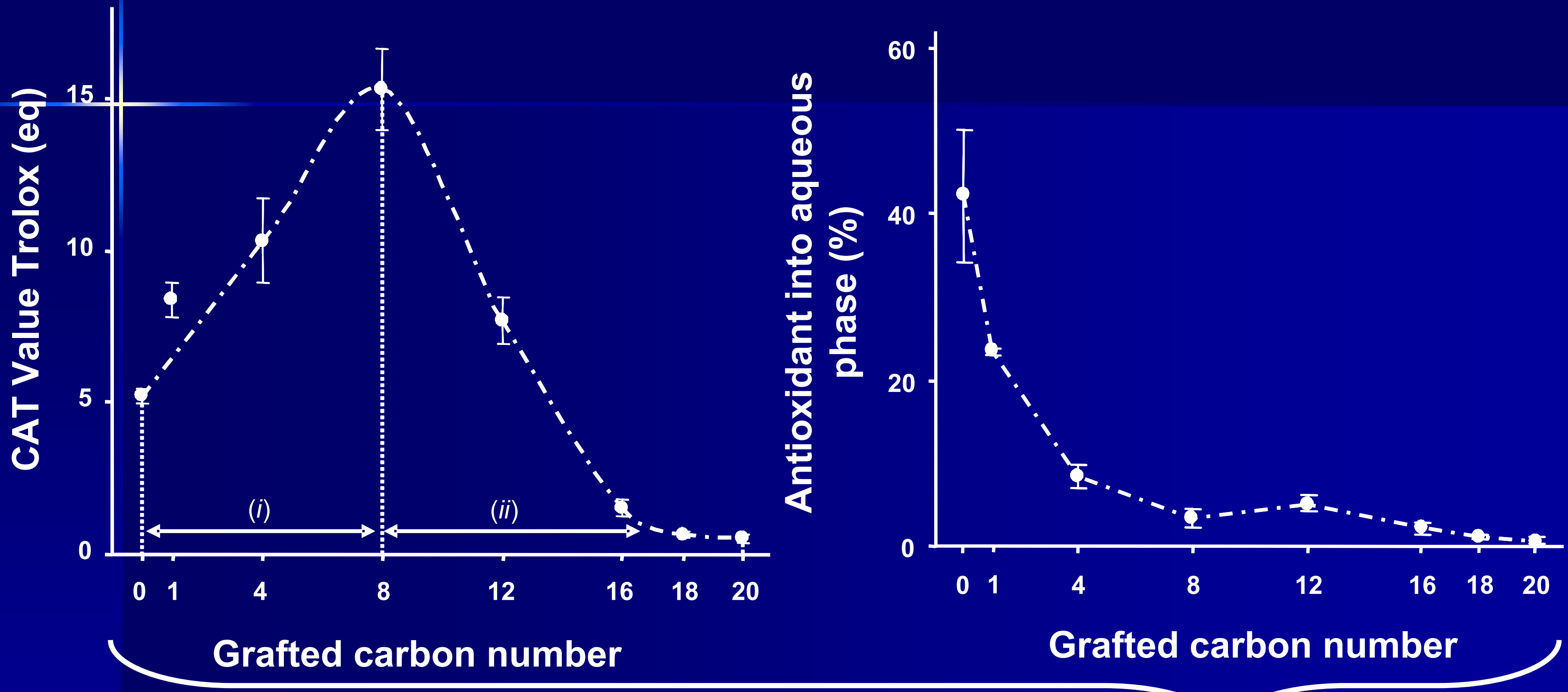
C) Carbon number > 16



Quantitative Evaluation of Antioxidant Capacity. Rosmarinic acid and its esters



Distribution and Antioxidant Capacity. Rosmarinic acide case



How much antioxidant ?

- at interface ?
- into oil phase ?

No relation with antioxidant distribution into aqueous phase

Conclusions

- Best performances of enzymatic lipophilization.
- Optimal chain length for antioxidant activity.
- Antioxidant activity in partial agreement with “Polar Paradox”.
- Relation between partition and antioxidant activity, but antioxidant activity is a multi-factorial phenomena.
- Substitution of synthetic antioxidants.
- Possible utilization as anticancer molecules.

THANK YOU FOR YOUR ATTENTION