Applications of enzymes in industry for bulk productions: Oleochemistry

Major biotransformations at industrial scale 1.

Fine-pharma chemistry

Production scale [tpy]	Product	Enzyme	Reactor	Company
> 1 000 000	high-fructose corn syrup (HFCS)	glucose isomerase	fixed-bed, IME	various
> 100 000	lactose-free milk	lactase	fixed-bed, IME	various
> 10 000	acrylamide	nitrilase	batch reactor	Nitto Co.
	cocoa butter*	lipase (CRL)	fixed-bed, IME	Fuji Oil
> 1,000				
	nicotinamide	nitrilase	3-stage batch	Lonza Guangzhou
	D-pantothenic acid	aldonolactonase		Fuji Pharma-ceuticals
	(S)-chloropropionic acid	lipase		Dow Chemical
	6-aminopenillanic acid	penicillin amidase	fixed-bed, IME	various
	7-aminocephalo-sporanic acid	glutaryl amidase	Kundl/Hoechst	
	aspartame®	thermolysin	soluble enzyme	Tosoh/DSM
	L-aspartate	aspartase	fixed-bed, IME	various
	D-phenylglycine	hydantoinase/ (carbamoylase)	resting cells	Kanegafuchi
	D-*p*-OH-phenyl-glycine	hydantoinase/ carbamoylase	resting cells	Recordati

Enzymatic bioprocessing of oils and fats

Determining the sustainability of enzyme processes with Life Cycle Assessment

David Cowan, Karen Margrethe Oxenbøll, and Hans Christian Holm

The industrial revolution, which started in Europe, has long since spread to much of the rest of the world and has raised living standards and brought prosperity to millions of people. However, t without consequences, not the least of whic industrialization to climate change. That this edged by a majority of climate change resea munity.

ronmental impact. In the case presented CA is applied to three process- within the oils and fats industry nthesis, degumming, and inter- ation) to compare the environ- mpact of the conventional and ic alternatives of each. In all the uts and outputs have been quan-

Oleochemistry

Lipases in the synthesis of esters:

- **Food ingredients**
- **Surfactants**
- **Emollients**
- **Biodiesel**

Lipases and phospholipases for the transformation of Fats and Oils

The basic oleochemicals are:
fatty acids (ca. 52%), the respective methyl esters (ca. 11%), amines (ca. 9%), and alcohols (ca. 25%).

Besides food applications, these are used for the production of important chemical products: **surfactants, lubricants and coatings** but also **biofuels.**

Vegetable Oils and fats production:
80 B Kg

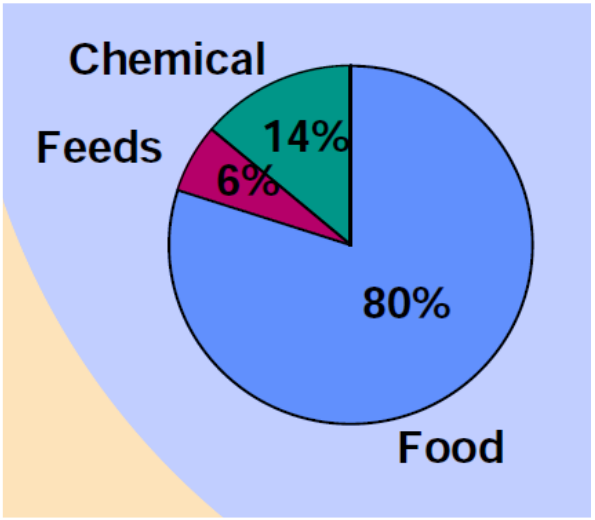
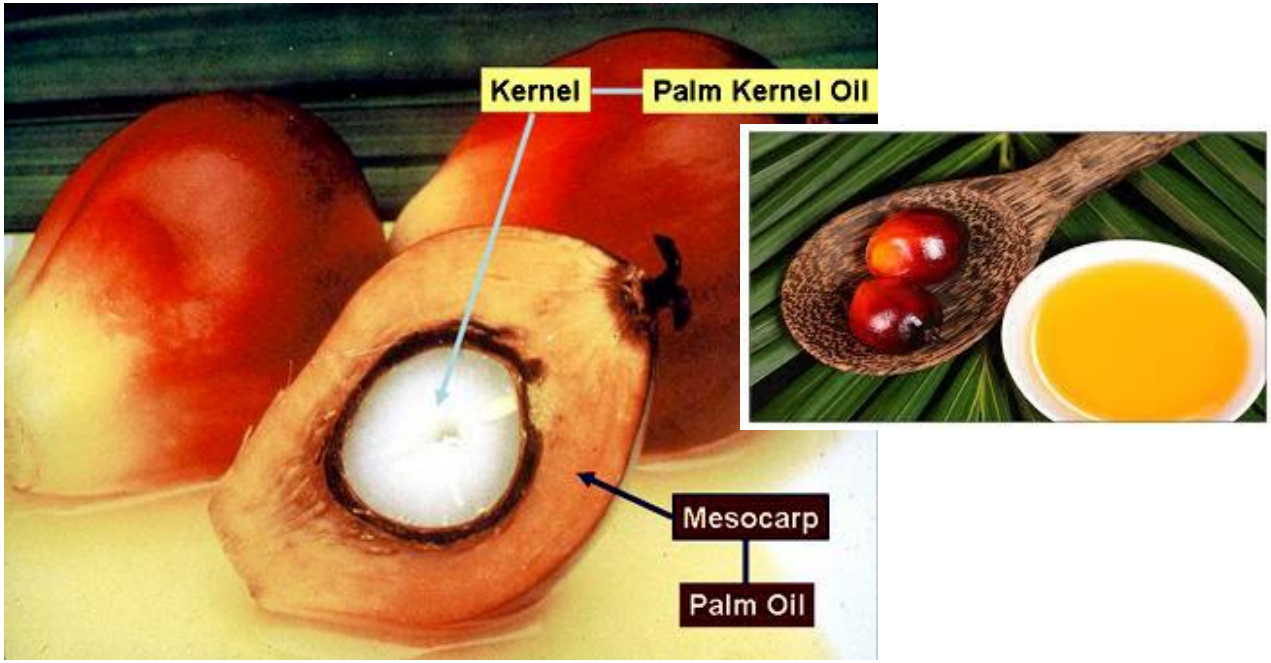


Figure 1. Production of oils and fats that are important as feedstock for the oleochemical industry in 1999/2000 and 2009/2010.[14,19]

- **palm kernel oil**
- **palm oil**



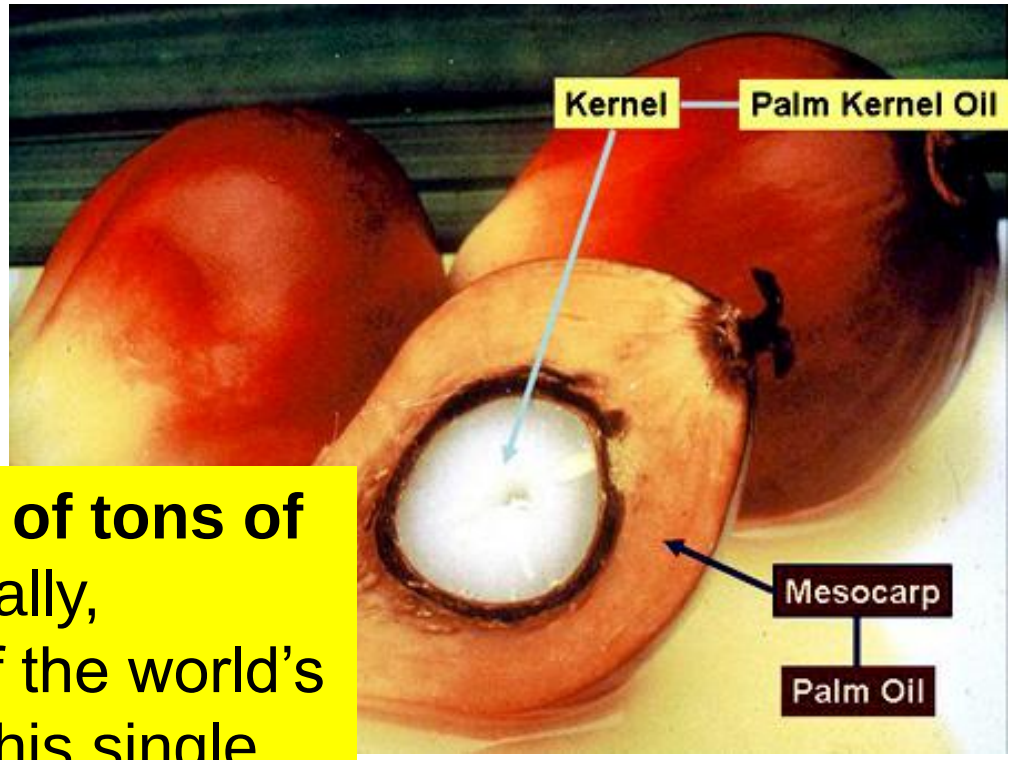
African oil palm
Elaeis guaineensis

Palm kernel oil:

- semi-solid at room T, more saturated than palm oil and comparable to coconut oil. Used in commercial cooking because of its low cost, and because it remains stable at high cooking temperatures and can be stored longer than other vegetable oils.(fewer C=C)
- is high in lauric acid which has been shown to raise blood cholesterol levels. Highly saturated vegetable fats

- **palm kernel oil,**
- **palm oil,**

African oil palm
Elaeis guaineensis



Palm oil: tens of millions of tons of palm oil is produced annually, accounting for over 30% of the world's vegetable oil production. This single vegetable oil is found in approximately 40-50% of household products in many developed countries. Palm oil can be present in a wide variety of products, including baked goods, shampoo, cosmetics, washing detergents and toothpaste.

Saturated fatty acids

palm mesocarp oil is 49% ,
palm kernel oil 81%
coconut 86%

Fatty acid content of palm kernel oil

Type of fatty acid	
Lauric saturated C12	48.2%
Myristic saturated C14	16.2%
Palmitic saturated C16	8.4%
Capric saturated C10	3.4%
Caprilic saturated C8	3.3%
Stearic saturated C18	2.5%
Oleic monounsaturated C18:1	15.3%
Linoleic polyunsaturated C18:2	2.3%
other	0.4%

Fatty acid content of palm oil

Type of fatty acid	
Mirystic saturated C14	1.0%
Palmitic saturated C16	43.5%
Stearic saturated C18	4.3%
Oleic monounsaturated C18	36.6%
Linoleic polyunsaturated C18	9.1%
Other/Unknown	5.5%

Phospholipases: enzymatic method as an alternative to the conventional method of alkaline degumming.

Enzymatic degumming of vegetable oils.

This process is used in oils and fats production to remove phosphatide gums from oil, which have an adverse effect on oil quality and stability.

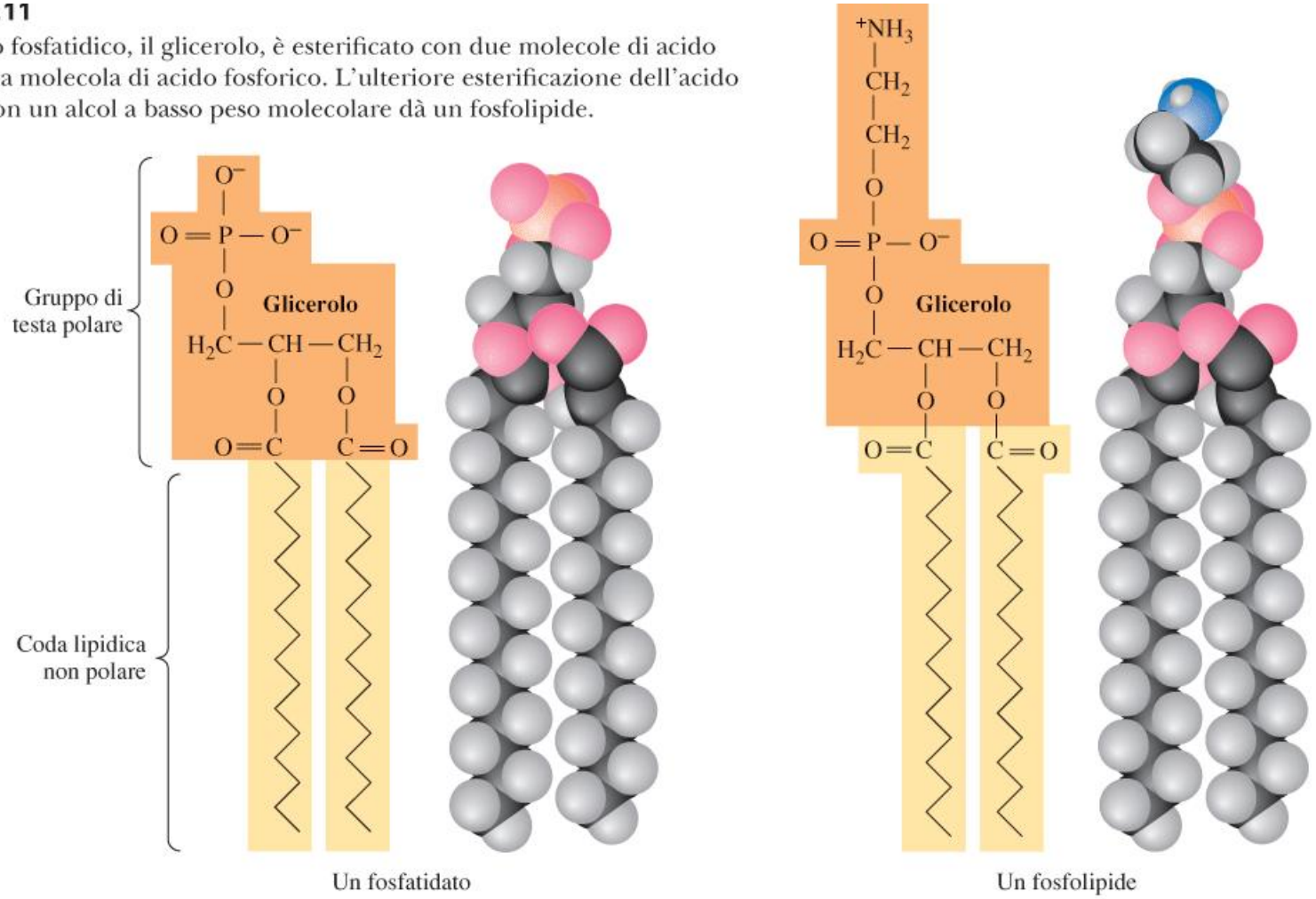
The affinity for water of the ester group determines the overall affinity for water of the phospholipid: the higher the water affinity, the higher the emulsification power. The relative affinity of phospholipids for water is usually called “hydratability.”

https://www.youtube.com/watch?v=sEGKAXlX9WM

https://www.youtube.com/watch?v=vq2Ge7hdMys

Figura 26.11

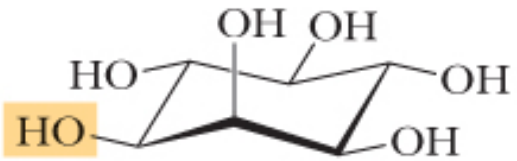
In un acido fosfatidico, il glicerolo, è esterificato con due molecole di acido grasso e una molecola di acido fosforico. L'ulteriore esterificazione dell'acido fosforico con un alcol a basso peso molecolare dà un fosfolipide.



Un fosfatidato

Un fosfolipide

Alcoli presenti nei fosfolipidi

Formula di struttura	Nome	Nome del fosfolipide
$HOCH_2CH_2NH_2$	Etanolammina	Fosfatidiletanolammina (Cefalina)
$HOCH_2CH_2\overset{+}{N}(CH_3)_3$	Colina	Fosfatidilcolina (Lecitina)
$HOCH_2CHCOO^-$ (con NH_3^+)	Serina	Fosfatidilserina
	Inositolo	Fosfatidilinositolo

Those phospholipids responsible for higher oil losses due to oil emulsion, , i.e. PC and PI, are usually designated as hydratable phospholipids (HP), while PA and PE salts of calcium (Ca), magnesium (Mg) and iron (Fe) are usually called nonhydratable phospholipids (NHP).

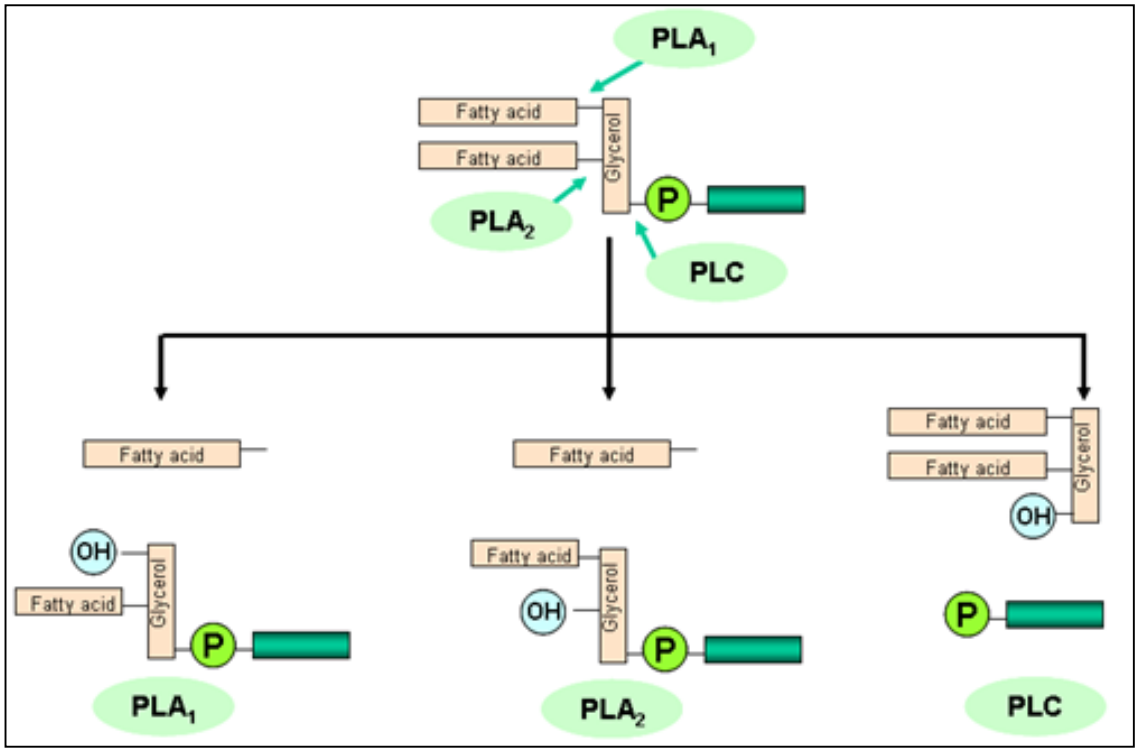
Relative rates of phospholipid hydration

Phospholipid	Relative rate of hydration
Phosphatidylcholine	100
Phosphatidylinositol	44
Phosphatidylinositol (calcium salt)	24
Phosphatidylethanolamine	16
Phosphatidic acid	8.5
Phosphatidylethanolamine (calcium salt)	0.9
Phosphatidic acid (calcium salt)	0.6

Compared with chemical processes, enzyme-catalyzed reactions are very selective, greatly reducing or eliminating the formation of undesired by-products. The enzymes active on phospholipids are called **phospholipases**.

The enzymes able to **remove fatty acids from the phospholipids** are conventionally named phospholipase A (PLA) (1 or 2, according to the position of the fatty acid removed from the glycerol backbone).

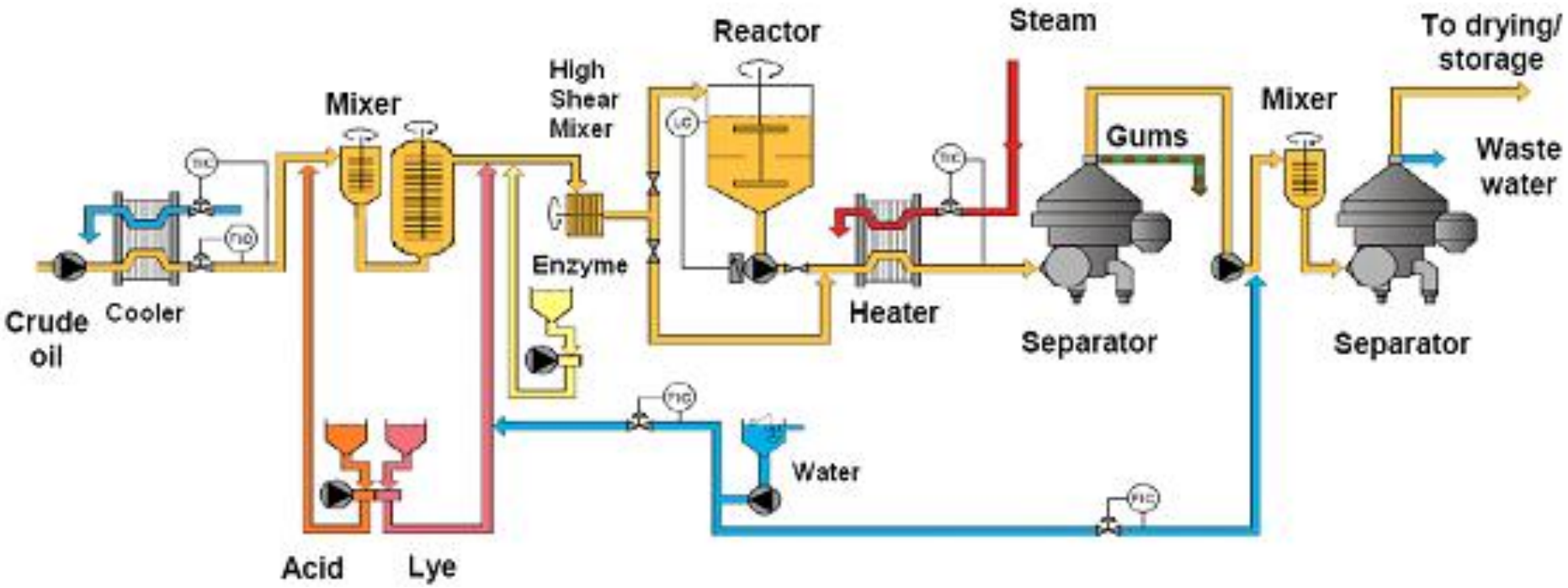
Phospholipase C (PLC) is able to **remove the "phospho-ester" group and generate a diglyceride for each phospholipid molecule reacted**.



Commercially available phospholipases for enzyme degumming			
Supplier	**Brand**	**Activity**	**Organism of origin**
AB Enzymes	Rohalase®MPL	PLA2	*Trichoderma reesei* [20]
Danisco	Lysomax®	PLA2	*Streptomyces violaceoruber*[21]
DSM	Gumzyme®	PLA2	*Aspergillus niger*[22]
Novozymes	Lecitase®10L	PLA2	Porcine pancreas [21]
Novozymes	Lecitase® Novo	PLA1	*Fusarium oxysporum* [15]
Novozymes	Lecitase® Ultra	PLA1	*Thermomyces lanuginosa* / *Fusarium oxysporum* [15]
Verenium	Purifine™ PLC	PLC	*Pichia pastoris*[23]

1: **Use of Phospholipase A**

•Adjusting the temperature the enzyme reaction;
•Adding citric acid and retention of the oil with acid for a period of time;
•Adding caustic soda to **optimal pH for the enzyme**
•**Adding enzyme and water** to the oil, via mixers– gums re-circulated here when using the enzyme;
•**Reacting in the reaction tank** (up to six hours);
•Heating the oil to the optimal temperature for separation in the centrifuge; and
•Separating lyso-phosphatides from the oil in the centrifuge



https://www.youtube.com/watch?v=9hO724frVWc

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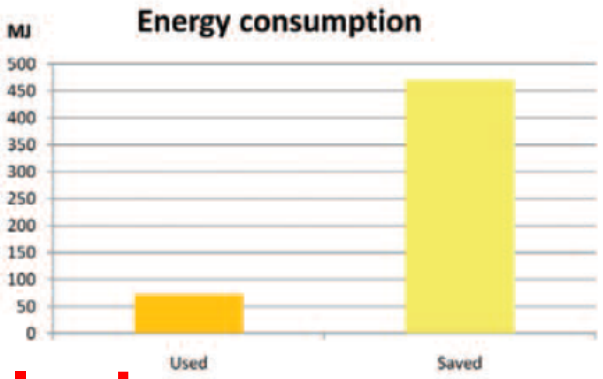
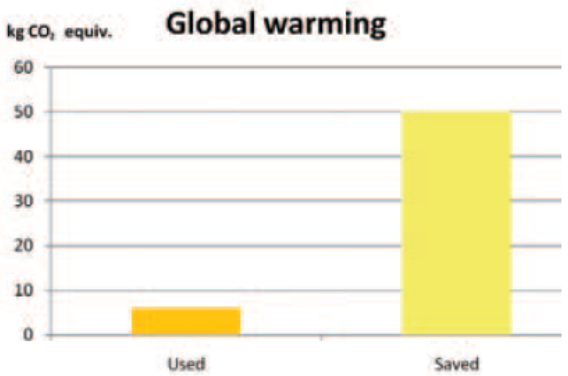
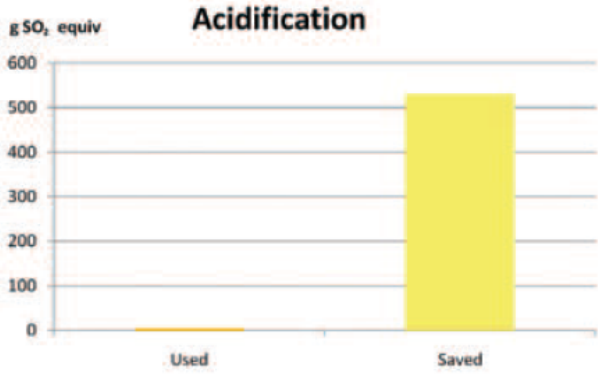
https://www.youtube.com/watch?v=f3Yx6Z0YRsk

Life cicle analysis
LCA







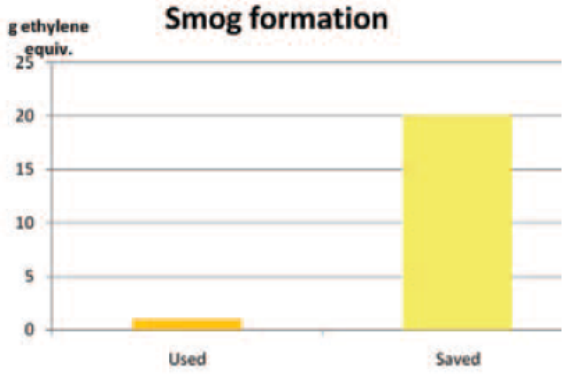


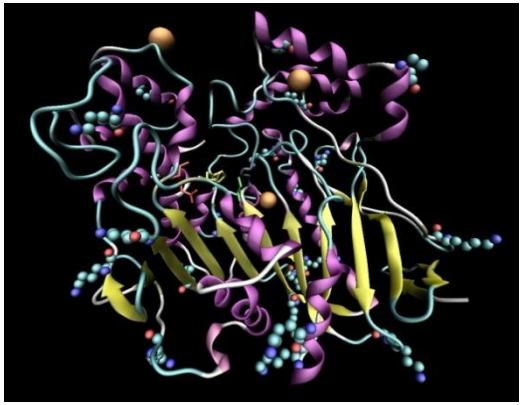
FIG. 2. *Environmental savings when using an enzymatic process for degumming of vegetable oil (per metric ton of refined soybean oil).*

TABLE 1. Identification of savings in enzymatic degumming

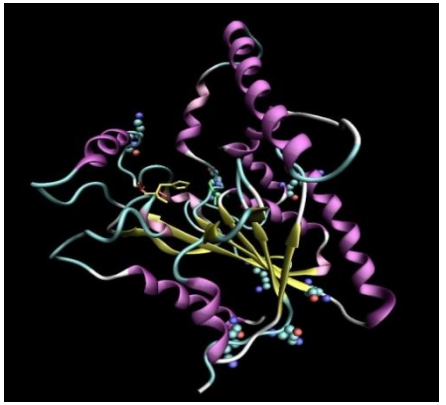
	Global warming	Acidification	Nutrient enrichment	Smog formation	Fossil energy
Vegetable oil savings	50%	50%	90%	80%	35%
NaOH savings	20%	10%	—	10%	25%
Phosphoric acid savings	—	10%	10%	—	5%
Byprod. treatment (incl. heat production)	20%	30%	—	10%	35%

Lipases for hydrolysis, esterification/transesterification:

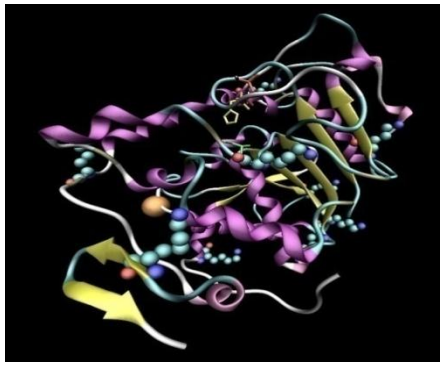
Burkholderia cepacia (Ps. cepacia) (33 kDa)



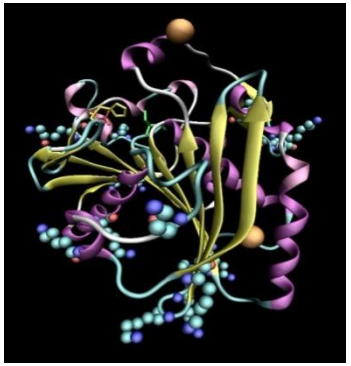
Candida rugosa (o cylindracea) (57 kDa)



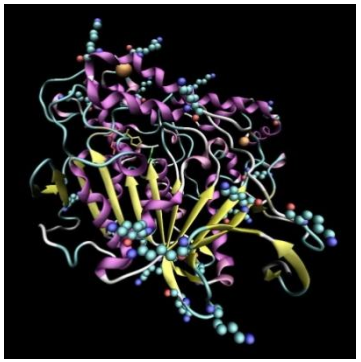
Pseudomonas aeruginosa (33 kDa)



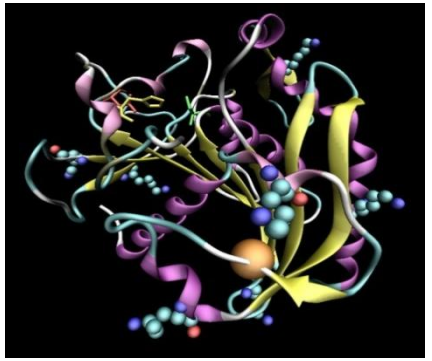
Candida antarctica B (33 kDa)



Rhizopus niveus (30 kDa)

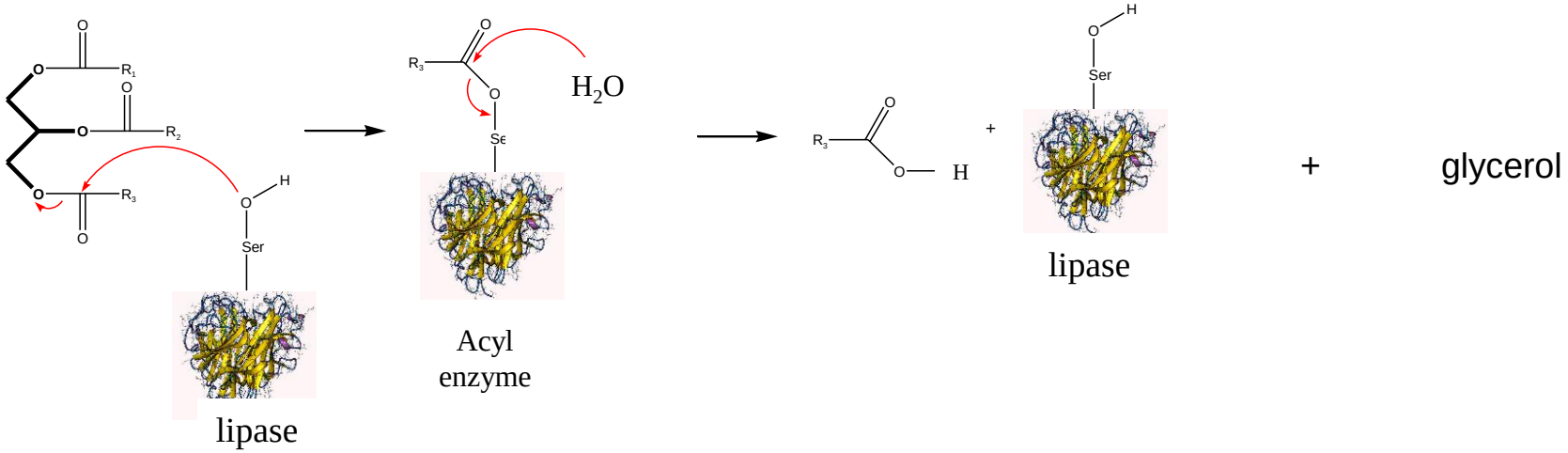


Geotrichum candidum (60 kDa)



Humicola lanuginosa (60 kDa)

Lipase hydrolyze (digest) triglycerides at mild conditions



Advantages of industrial applications (coco nuts oil, olive oil…):

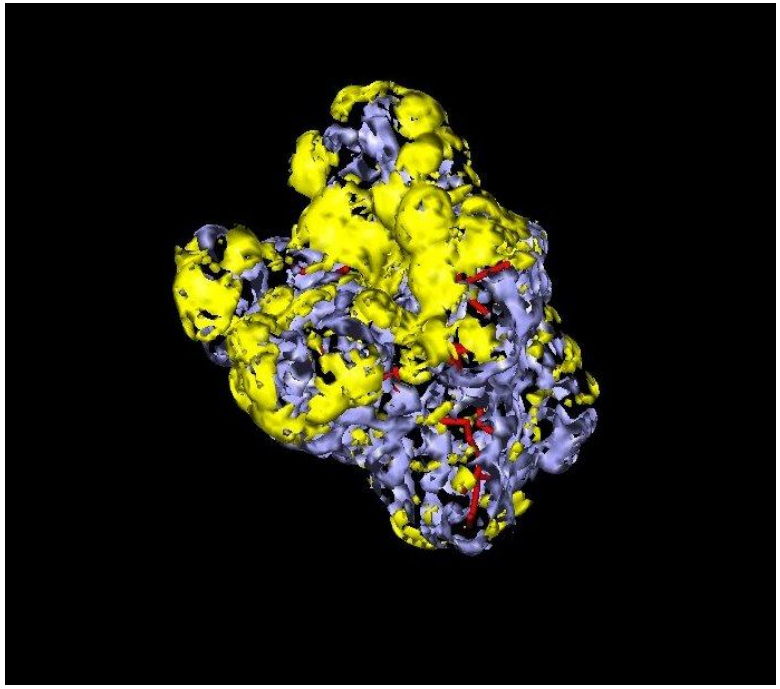
a) reduction of wastes and side products
b) mild and neutral reaction conditions leading to highest quality products
c) higher purity of the glycerol obtained as secondary product

Table 3. Important commercially available lipases.

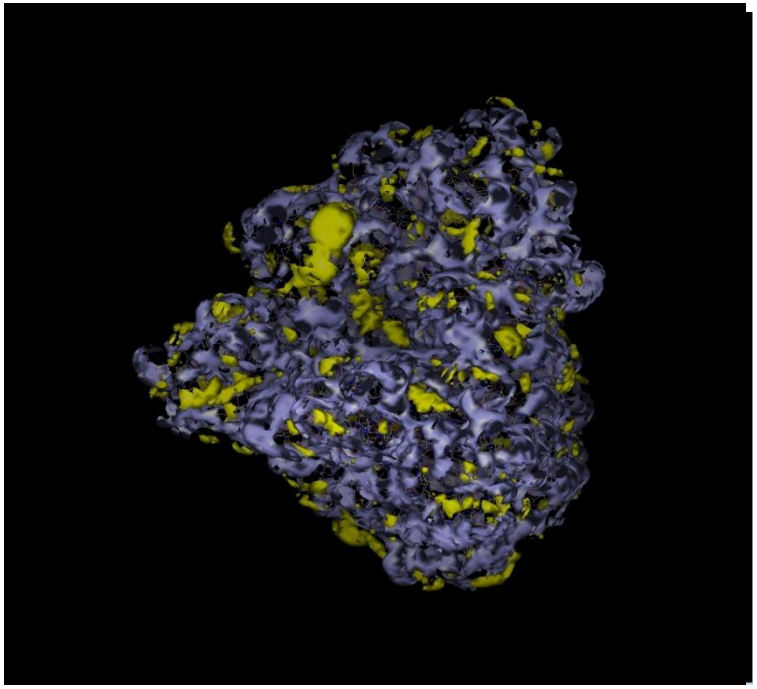
Origin	Code[a]	*M* [kDa] (rounded)	Specificity (remarks)	Applications
of mammalian origin				
human pancreatic lipase	HPL	50	*sn*-1,3	
human gastric lipase	HGL	50	*sn*-3 (acid-stable)	
porcine pancreatic lipase	PPL	50	*sn*-1,3	organic synthesis, digestive aid
guinea pig pancreatic lipase	GPL-RP2	48	*sn*-1,3 (phospholipase A1 activity)	
of fungal origin				
Candida rugosa	CRL	60	nonspecific	organic synthesis
Candida antarctica B	CAL	60	*sn*-1,3	organic synthesis
Geotrichum candidum	GCL	60	cis-Δ^9 (unsaturated fatty acids)	oleochemistry
Humicola lanuginosa	HLL	30	nonspecific	detergents
Rhizomucor miehei	RML	30	*sn*-1,3	cheese manufacturing
Aspergillus oryzae	AOL			cheese manufacturing
Penicillium camembertii	PEL	30	*sn*-1,3	monoglycerides
Rhizopus delemar	RDL	41	*sn*-1,3 (phospholipase A1 activity)	oleochemistry
Rhizopus oryzae	ROL	41	*sn*-1,3 (phospholipase A1 activity)	oleochemistry
Rhizopus arrhizus	RAL	41	*sn*-1,3 (phospholipase A1 activity)	oleochemistry
of bacterial origin				
Pseudomonas glumae	PGL	33	nonspecific	detergent enzyme, organic synthesis
Burkholderia cepacia	PCL/BCL	33	nonspecific	organic synthesis
Pseudomonas pseudoalcaligenes	PPL	33	*sn*-1,3	detergents
Pseudomonas mendocina	PML	33	*sn*-1,3	detergents
Chromobacterium viscosum	CVL	33	*sn*-1,3	organic synthesis
Bacillus thermocatenulatus	BTL-2	43	*sn*-1,3 (thermoalkalophilic)	
Fusarium solani (hydrolyzes cutin)	FSL	22		detergents

[a] Other appreviations for lipases used in this article: PSL (*Pseudomonas species* lipase), PFL (*Pseudomonas flourescens* lipase), HLL (human lipoprotein lipase), LPL (lipoprotein lipase). Lipases can be obtained commercially from many suppliers. Important original producers are Novo-Nordisk (Baegsvard, Denmark), Genencor International B. V. (Delft, The Netherlands), Boehringer-Mannheim (Mannheim, Germany), and Amano Co. (Nagoya, Japan).

- Lipases are proteins with wide hydrophobic areas on the surface
- Lipases have evolved to be active on insoluble and hydrophobic substrates



A lipase: large hydrophobic surface

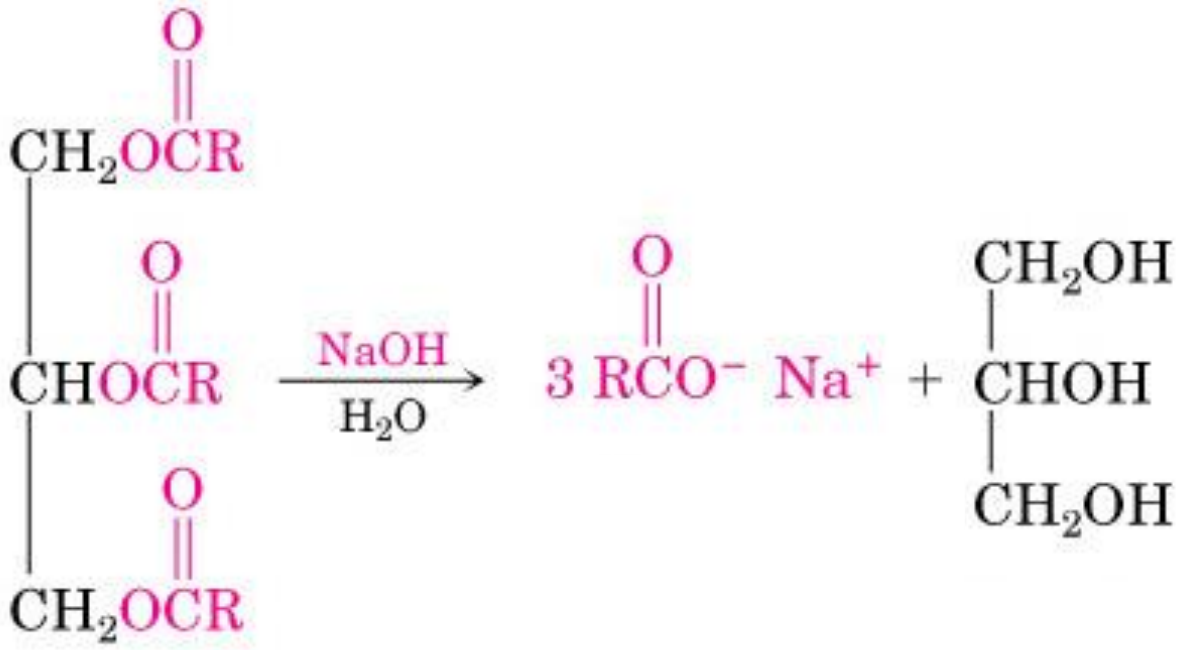


A general hydrolytic enzyme (amidase): hydrophylic surface

hydrophobic

hydrophilic

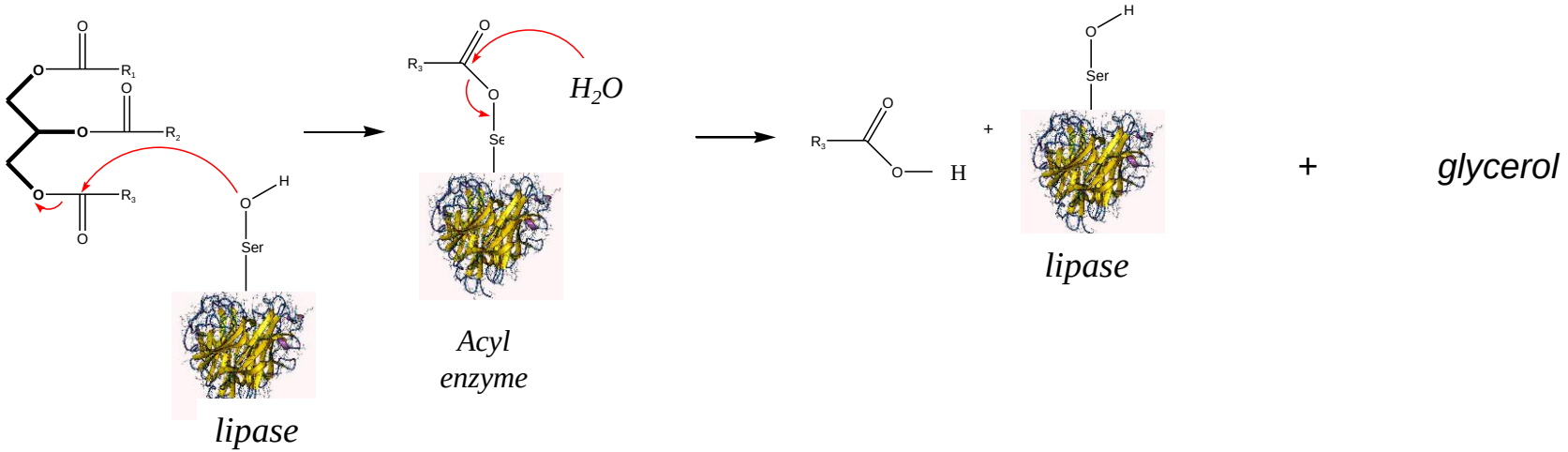
The chemical hydrolysis of oils requires strong basic reagents



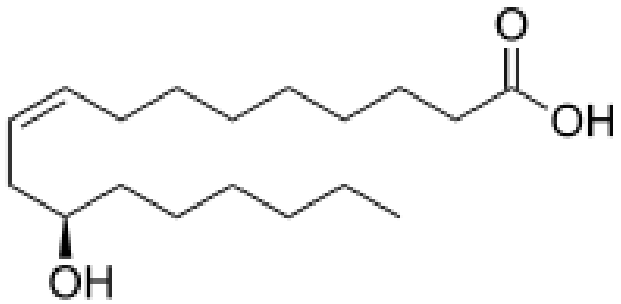
Triglyceride

Use of lipases in the transformation of fats and oils: Hydrolysis

This process employs the ability of lipases to hydrolyze, in the presence of water, the lipids so as to obtain fatty acids and glycerol, molecules that both have important industrial applications. For example, fatty acids are used for soap production. Lipases used for this purpose are those from *Candida rugosa*, from castor seed and *Pseudomonas fluorescens.*



Enzymatic hydrolysis can be used to obtain unstable fatty acids from oils containing unsaturated fatty acids. This is generally difficult to achieve by conventional means because of the high temperatures (200-240 ° C) and pressures used, which could lead to undesired lipid oxidation. This is demonstrated by the production of ricinoleic acid, a valuable starting material for the production of a wide variety of technical products: ricinoleic acid can not be obtained from castor oil with conventional processes due to side reactions, such as dehydration, interesterification, etc. These can be avoided by using a lipase present in castor seeds and used for the hydrolysis of castor oil.



ricinoleic acid

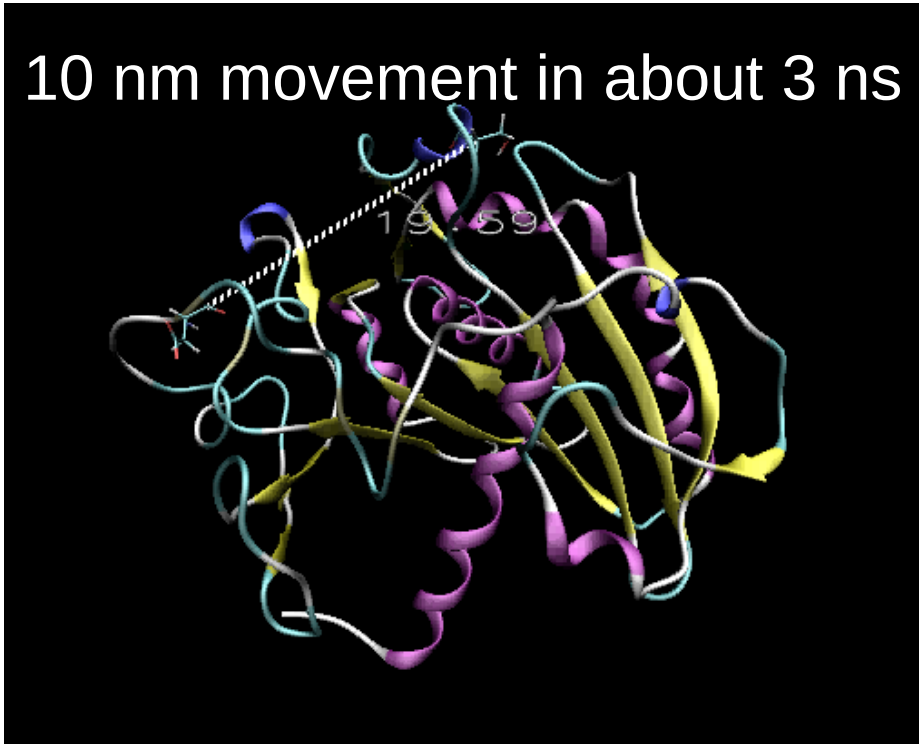
In contrast, the enzymatic cleavage of triglycerides is carried out at a pressure and ambient temperature (40-60 ° C), allowing a lower energy cost. The overall cost of the enzymatic process determines an economic advantage due to the fact that the reaction plants do not have to be very resistant to corrosion nor resist very aggressive reaction conditions.

Products that derive from enzymatic bioprocesses also have a better odor and color and are usually more pure, because secondary reactions with respect to conventional processes are avoided thanks to the selectivity of lipases and to the mild environmental conditions. This has the advantage of further reducing costs, thanks to the reduction in the number of extraction and purification procedures, furthermore the lower reaction temperatures guarantee less thermal degradation.

Lipases in organic media are generally used immobilized in order to prevent protein aggregation and to maximize dispersion/accessible surface

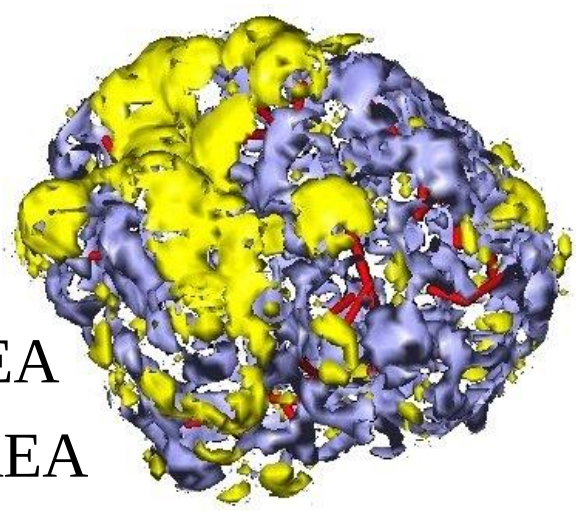
Lipases modify their **conformations** while approaching the triglycerides.

The hydrophobic active site becomes exposed and accessible

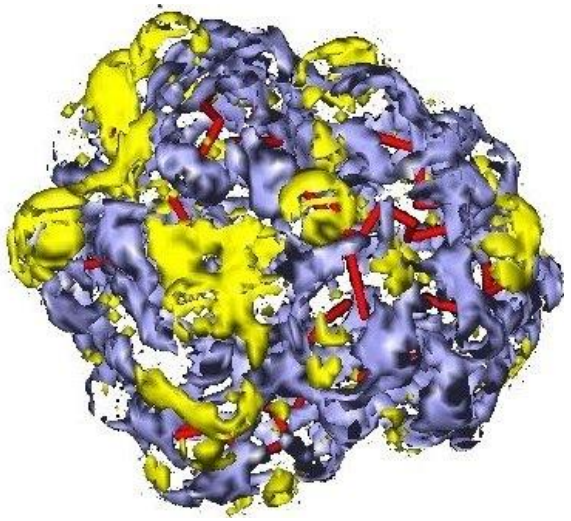


HYDROPHILIC AREA

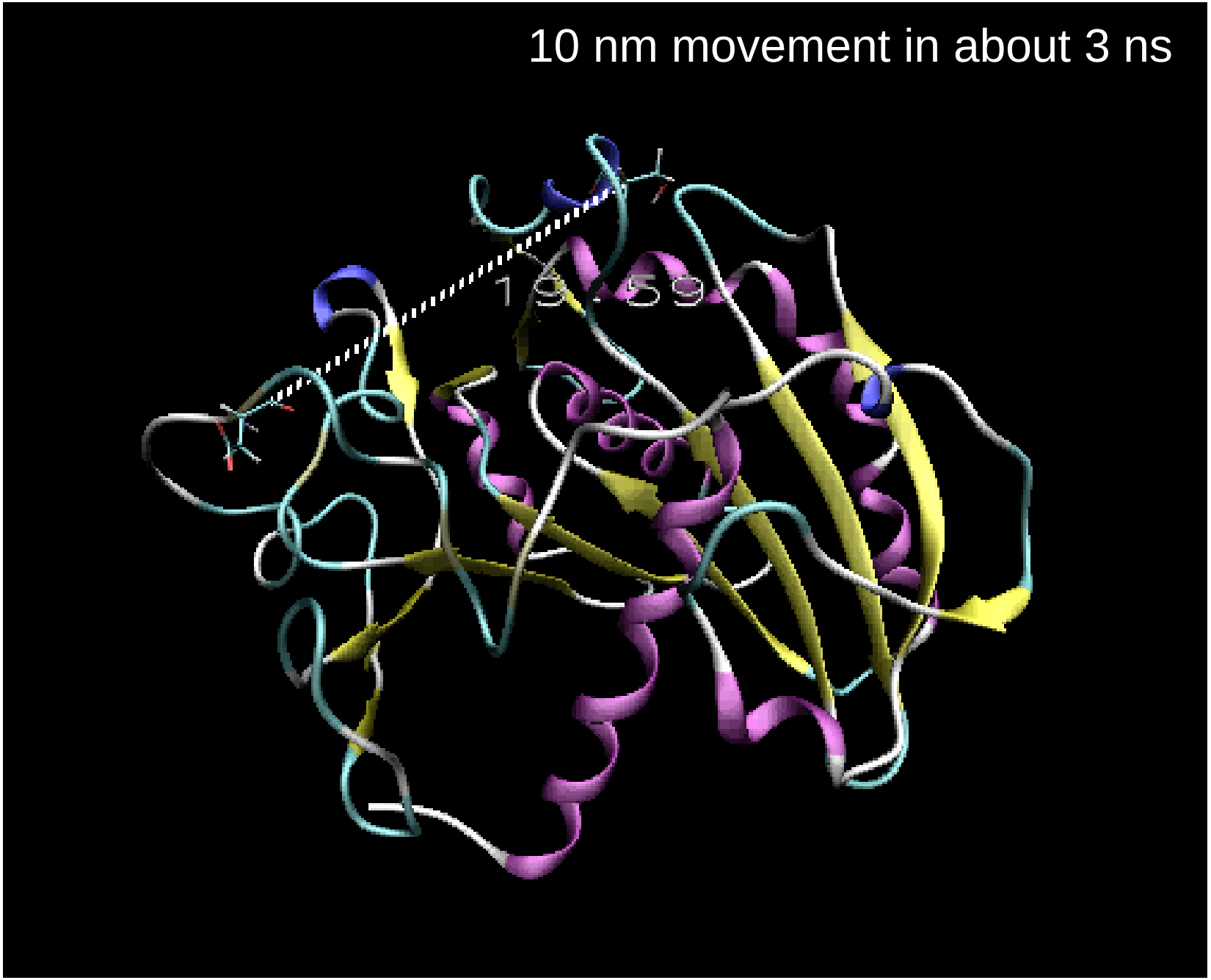
HYDROPHOBIC AREA



OPEN CONFORMATION

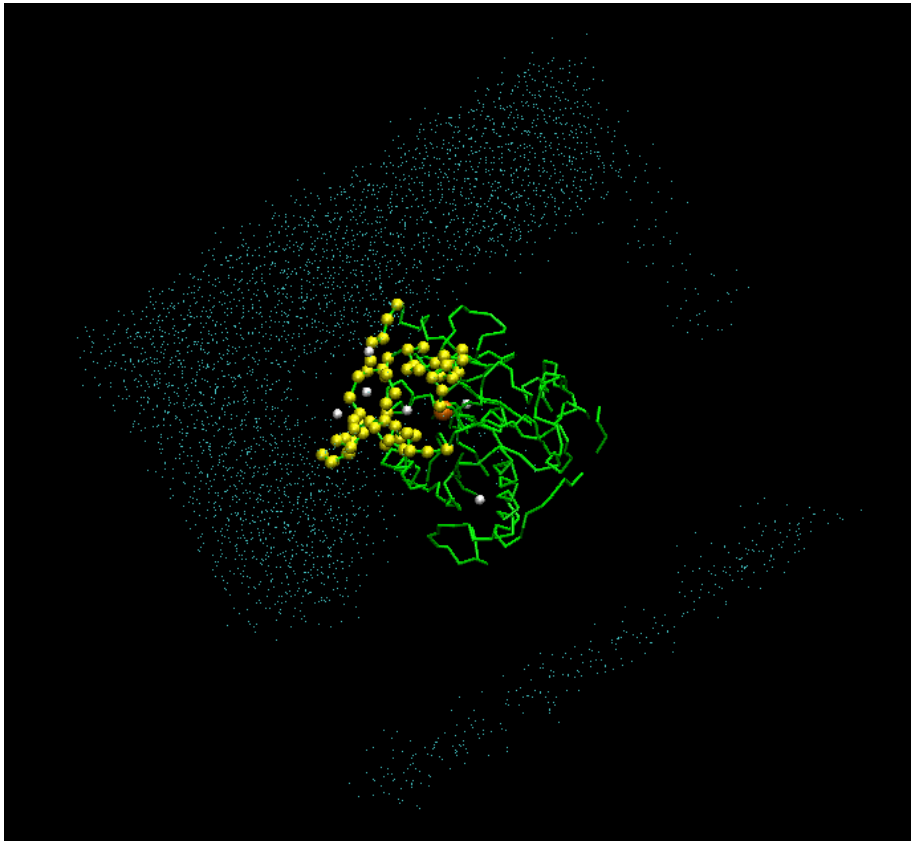


CLOSED CONFORMATION


10 nm movement in about 3 ns
19.59

Lipases are active at the interface oil/water: the hydrophobic surface points towards the hydrophobic phase

Simulation of Water-Octanol Interface

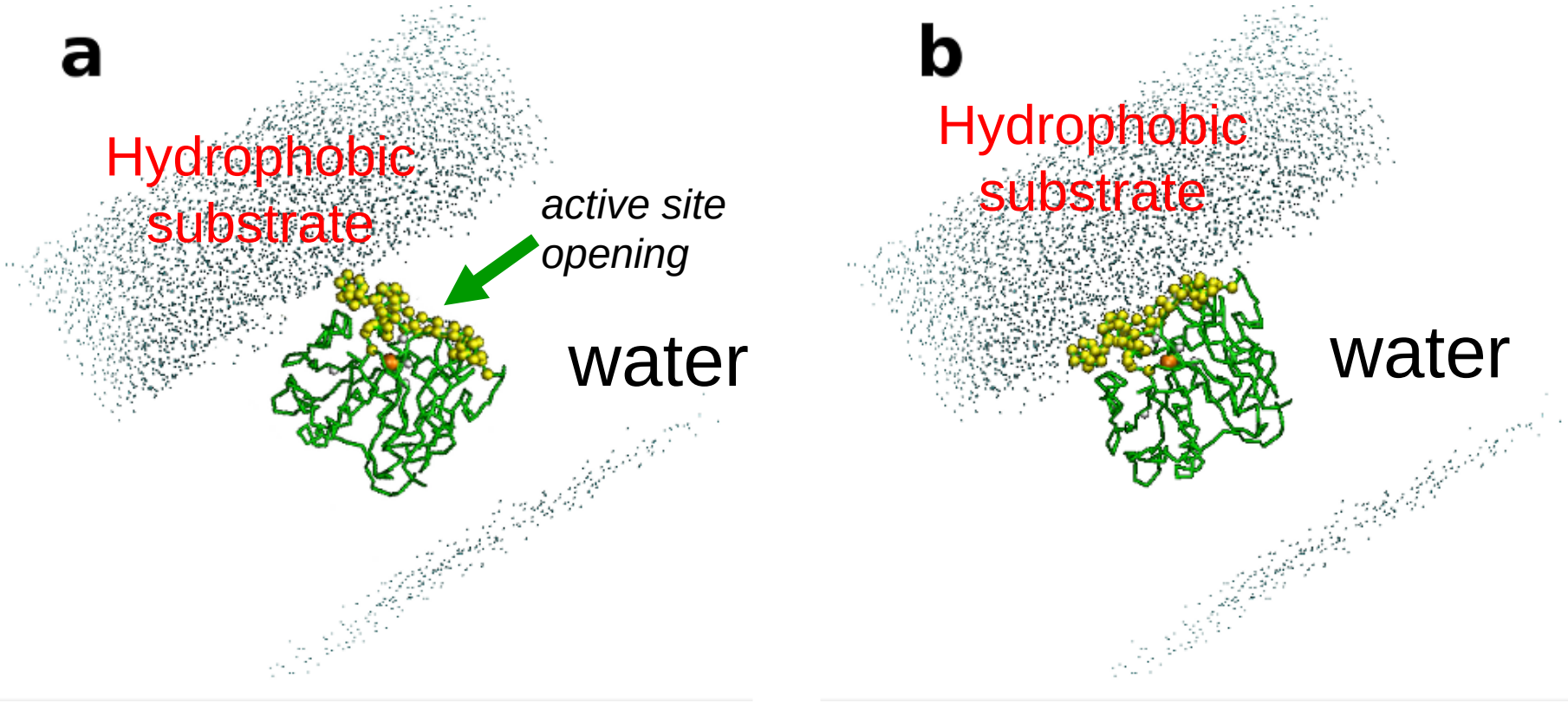


GROMACS
(MARTINI)

Martini FF - Protein

Pseudomonas cepacia lipase

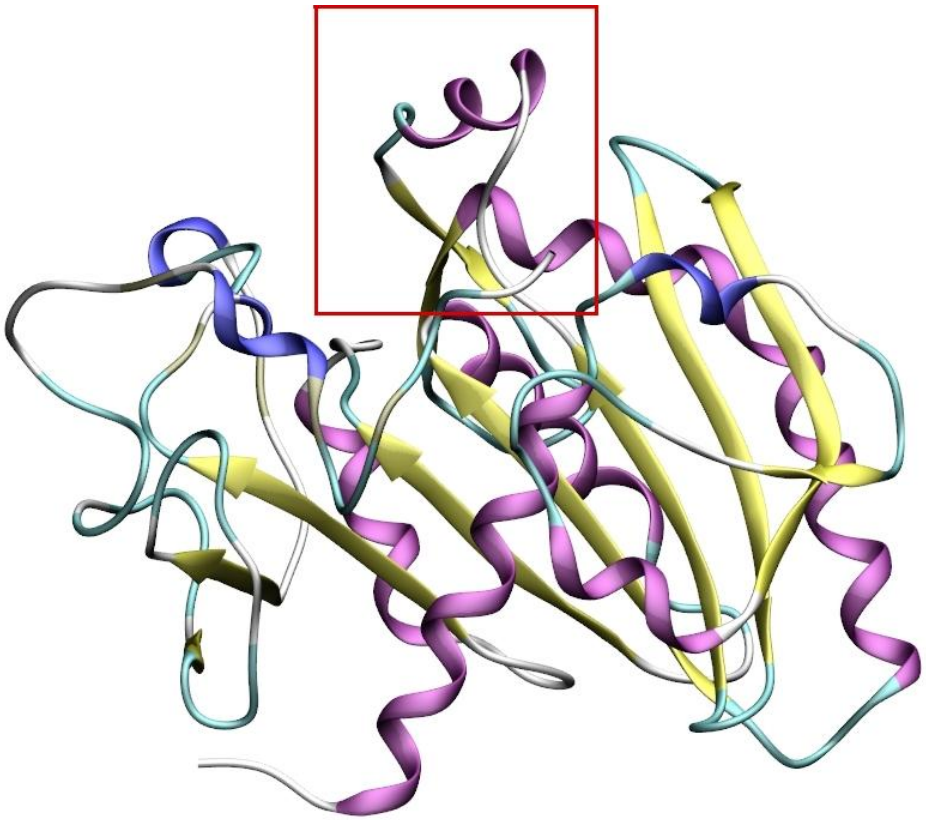
Lipases approach and hydrolyze insoluble hydrophobic substrates

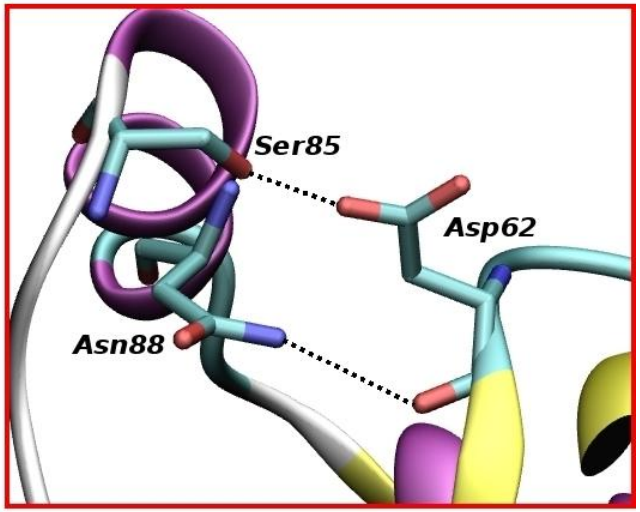


Molecular dynamics simulations of a lipase in a water-oil biphasic system (Martini software)

Mechanism and conformational modification (Lipases activation)

LID Stabilization in hydrophobic environment – open conformation





Humicola lanuginosa LIPASE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Cofactors
Metals/Ions
Activating Compounds
Ligands
Biochemicals Reactions Aligned NEW
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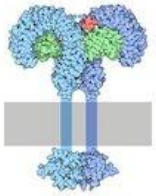

Molecule of the Month

Toll-like Receptors

The world is filled with bacteria and viruses, all eager to infect our cells. We have two lines of defense against this constant assault. Our first defense is the innate immune system, which stands guard against the most common attackers and mounts a quick defense when they are found. This innate system is found widely in animals, plants, and fungi, and for most, is the only line of defense.

Full Article

Protein Structure Initiative Featured System

Superbugs and Antibiotic Resistance

Antibiotics were used as weapons to fight bacteria long before Alexander Fleming discovered penicillin. Natural antibiotics like penicillin are made by fungi and other organisms to protect themselves, and as you might expect, bacteria have found many ways to avoid

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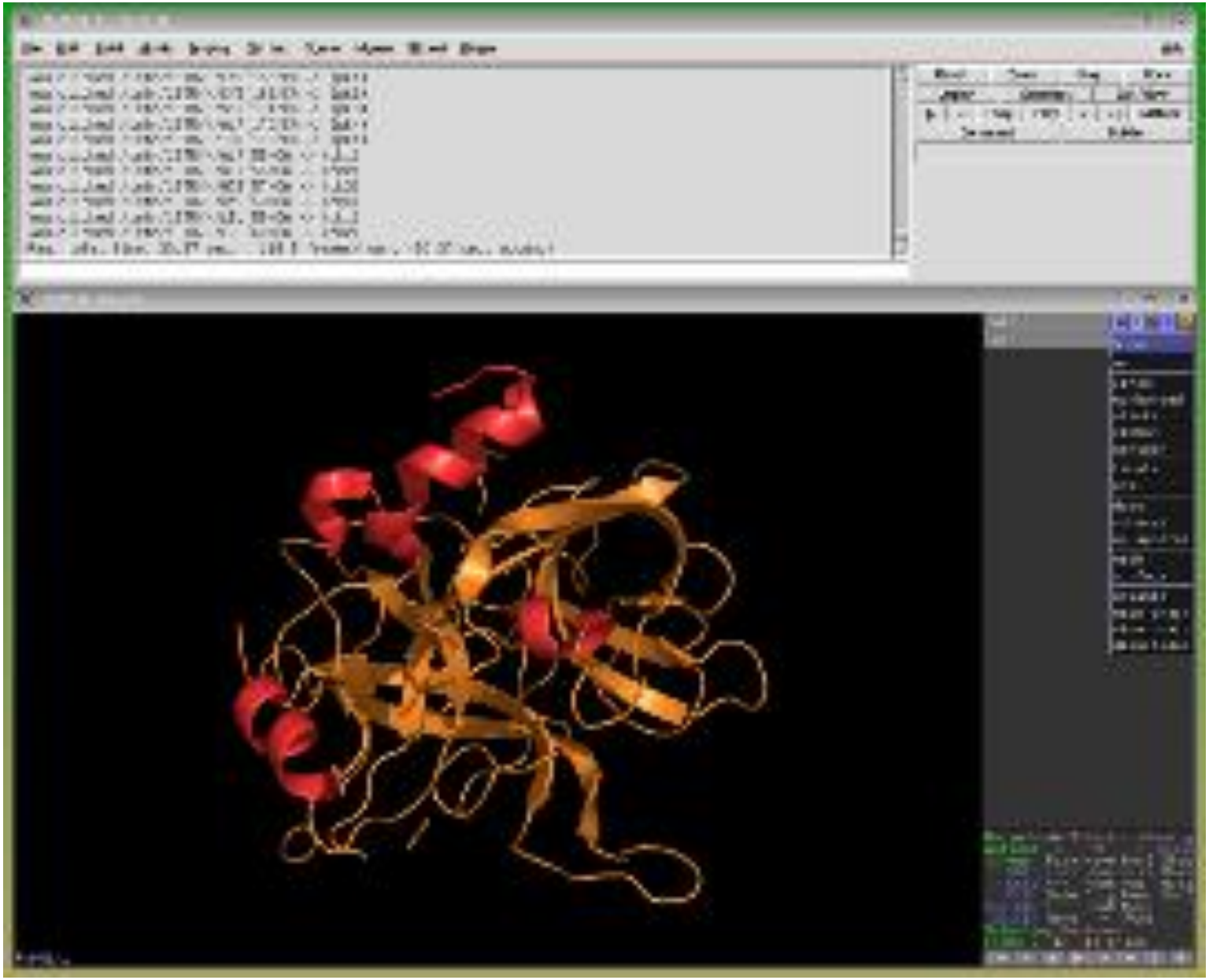


Oleochemistry

Lipases in the synthesis of esters:

- **Food ingredients**
- **Surfactants**
- **Emollients**
- **Biodiesel**

Surfactants

(i.e. surface-active agents with an amphiphilic structure) are mandatory ingredients of almost all cosmetic products for emulsification and alteration of foaming properties.

Ester-based surfactants, consisting of a medium to long chain fatty acid and a hydrophilic head group.

Depending on the length and saturation degree of the fatty tail and the choice and size of the hydrophilic head (typically glycerol, sorbitol or glucosides) employment for stabilisation of water-in-oil (W/O) lotions or oil-in-water (O/W) emulsions is possible.

The specific performance in this regard is described as the hydrophile–lipophile balance (HLB) value.

Of particular importance are **partial glycerol esters** (e.g. glycerol mono or dilaurate, which represents one of the oldest industrially produced emulsifiers for cosmetic application), inter alia because of their unique property of interacting with polysaccharides and polypeptides changing the rheological properties of the emulsion, and sugar esters, (e.g. sorbitan monostearate) due to their excellent surface-active properties.

Sugar esters based on short chain alkyl glucosides and fatty acids have been intensively investigated as targets for lipase catalysis due to the overall low (regio-)selectivity of chemical methods towards the numerous hydroxy groups in sugars .

Mainly two strategies for the lipase-catalysed synthesis of sugar esters can be distinguished. The first was based on the use of organic solvents suitable for the solubilisation of both substrates (typically dimethylsulphoxide, dimethyl formamide or pyridine), while the second relied on modification of the sugar moiety followed by solvent-free esterification with molten fatty acids. Although the first procedure is more straightforward, the reaction kinetics are such that the overall productivity is poor. Furthermore, use of solvents is highly unwanted in the cosmetics industry. For these reasons, the second methodology is more attractive.

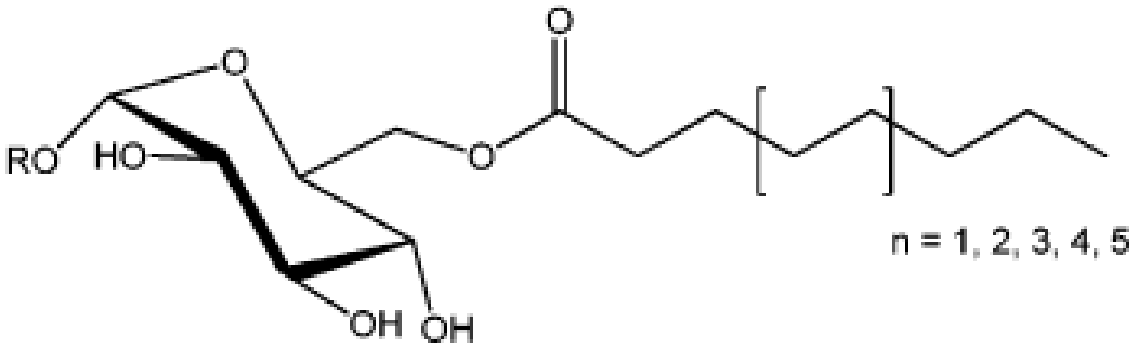
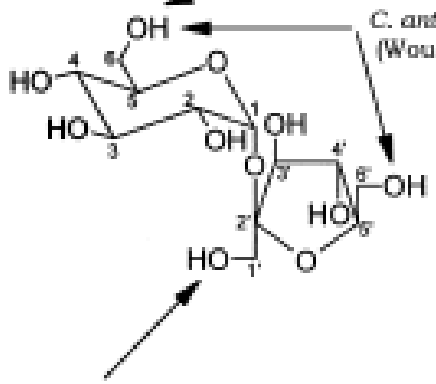


Fig. 4 Schematic of glycoside esters described by Björkling *et al.*;[63] R = short alkyl chain.

Sucrose

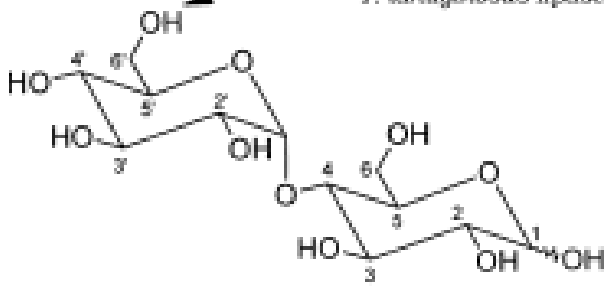
Pseudomonas sp. lipase (Rich *et al.*, 1995)
M. miehei lipase (Kim *et al.*, 1998)
T. lanuginosus lipase (Ferrer *et al.*, 1999)

C. antarctica lipase (Woudenberg *et al.*, 1996)



Subtilisin (Riva *et al.*, 1988; Cruces et al., 1992; Soedjak & Spradlin, 1994; Rich *et al.*, 1995; Polat *et al.*, 1997)
Protease N (Carrea *et al.*, 1989)

Subtilisin (Riva *et al.*, 1988)
B. fulva lipase (Ku *et al.*, 1995)
C. antarctica lipase (Woudenberg et al., 1996)
T. lanuginosus lipase (Ferrer *et al.*, 2000a)

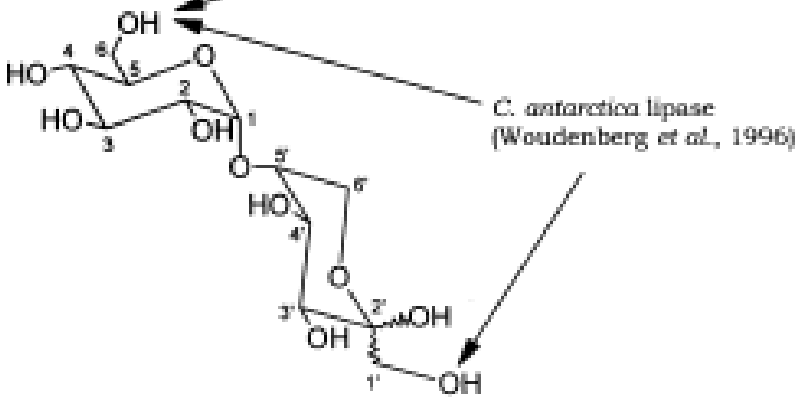


Maltose

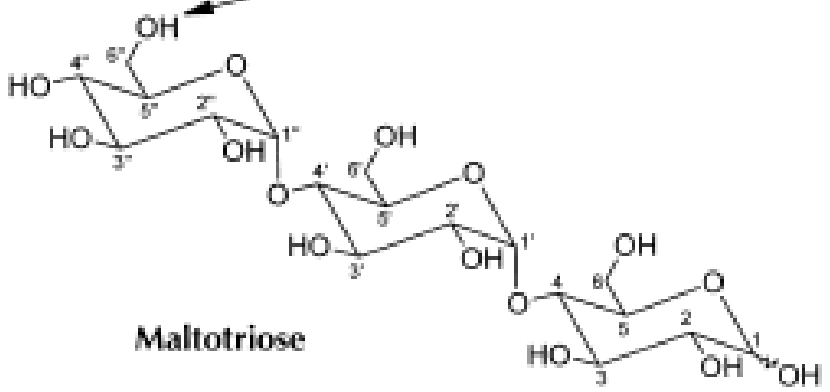
Leucrose

T. lanuginosus lipase (Ferrer *et al.*, 2000a)

C. antarctica lipase (Woudenberg *et al.*, 1996)



Subtilisin (Riva *et al.*, 1988)
T. lanuginosus lipase (Ferrer *et al.*, 2000a)



Maltotriose

Cosmetic industries

The world market for **cosmetics and toiletries** accounts to about 200 billion Euro

(source: Euromonitor International Database)

In 2010,

-cosmetics market within the EU-27: 67 bn €

- US 38 bn €

- Japan 30 bn €

(source: Colipa Annual report 2010).

Most cosmetic products have a **lifespan of less than five years** and manufacturers reformulate 25% of their products every year. They need to **improve products constantly** in order to stay ahead in a highly competitive market where more choice and ever greater efficacy are expected by the consumer.

Cosmetica

I principali Gruppi chimici italiani – Anno 2020

	Vendite mondiali	Produzione in Italia	Addetti mondiali	Addetti in Italia
	(milioni di euro)			
1. Versalis	3.381	2.545	5.295	4.304
2. Gruppo Mapei	2.772	837	10.495	2.357
3. Gruppo Bracco	1.199	656	2.726	600
4. Radici Group	1.019	621	3.100	1.562
5. Gruppo SOL	974	420	4.613	1.248
6. P & R Group	864	792	3.751	2.927
7. COIM Group	780	399	1.032	480
8. Gruppo SIAD	692	507	1.980	1.214
9. Polynt Group	671	488	1.282	944
10. Gruppo Sapio	629	532	2.268	1.405
11. Gruppo Intercos	604	355	5.191	1.697
12. Gruppo Sodalis	585	350	891	696
13. Gruppo Colorobbia	526	200	2.031	679
14. Italmatch Chemicals	518	141	928	241
15. FIS	516	500	1.849	1.849
16. Gruppo Zobele	509	78	5.451	273
17. Gr. Sipcam-Oxon	461	279	946	446
18. Esseco Group	439	241	1.352	680
19. Gruppo Aquafil	437	170	2.695	798
20. Gruppo Lamberti	421	206	1.269	733
21. Gruppo Desa	326	324	436	417
22. Fluorsid Group	325	183	360	231
23. Novamont	286	286	479	466
24. FACI Group	237	94	464	198
25. Reagens	235	102	387	173
26. Indena/Gr. IdB Holding	217	177	875	565
27. Gruppo Metlac	216	216	188	180
28. 3V Partecipaz. Industriali	208	140	594	398
29. Alfa Parf Group	204	33	2.088	473
30. Sabo	173	173	148	148
31. Sadepan Chimica	170	122	183	138
32. Mirato Group	167	167	457	411
33. Gruppo Silvateam	156	111	697	277
34. Davines	153	153	597	384
35. Gruppo Coswell	152	152	405	324
36. Gr. Durante/TLD Hldg	152	150	392	375
37. Istituto Ganassini	152	85	390	145
38. Gruppo Bozzetto	152	56	509	191
39. AGF88 Holding	135	135	520	520
40. Pagliери	135	135	134	134
41. Lechler	127	110	565	370
42. Dipharma Francis	126	119	495	457
43. Renner	125	125	409	381
44. Gruppo Biolchim	125	118	414	286
45. Adriatica	114	91	222	162
46. Gruppo Isagro	110	110	329	253
47. Gruppo SOL.MAR.	108	104	164	164
48. Sacco System	108	101	376	348
49. ICAP-SIRA	104	102	266	256
50. Madel	104	98	140	140
51. Index	100	100	165	165

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Fonte: Federchimica sui dati forniti dalle imprese - associate e non - che hanno aderito all'indagine

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Immobilised lipases in the cosmetics industry

Marion B. Ansorge-Schumacher*[a] and Oliver Thum*[b]

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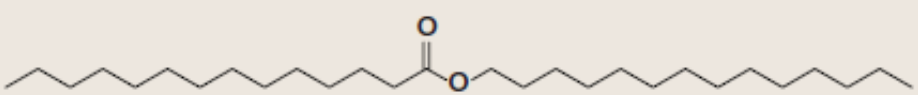
Commercial products for personal care, generally perceived as cosmetics, have an important impact on everyday life worldwide. Accordingly, the market for both consumer products and specialty chemicals comprising their ingredients is considerable. Lipases have started to play a minor role as active ingredients in so-called 'functional cosmetics' as well as a major role as catalysts for the industrial production of various specialty esters, aroma compounds and active agents. Interestingly, both applications almost always require preparation by appropriate immobilisation techniques. In addition, for catalytic use special reactor concepts often have to be employed due to the mostly limited stability of these preparations. Nevertheless, these processes show distinct advantages based on process simplification, product quality and environmental footprint and are therefore apt to more and more replace traditional chemical processes. Here, for the first time a review on the various aspects of using immobilised lipases in the cosmetics industry is given.

Lipases in cosmetic sectors

Sustainability That Gets Under the

DR. OLIVER THUM

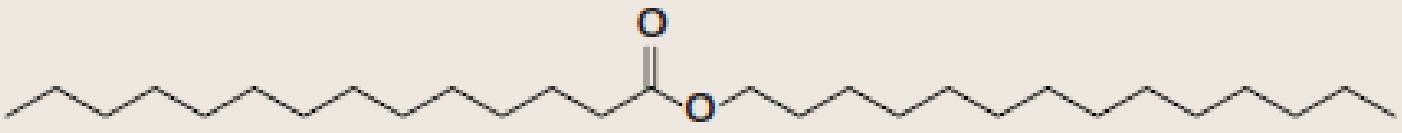
Evonik Industries is the only company worldwide that offers biotechnologically produced emollient esters for the cosmetics industry. Compared to the chemical production process, the biotechnological variant boasts extraordinarily good selectivity, mild reaction conditions, and high product pu
For the first time, researchers at Evonik have used a life c
quantitatively record and evaluate the advantages of bioc
of myristyl myristate production.

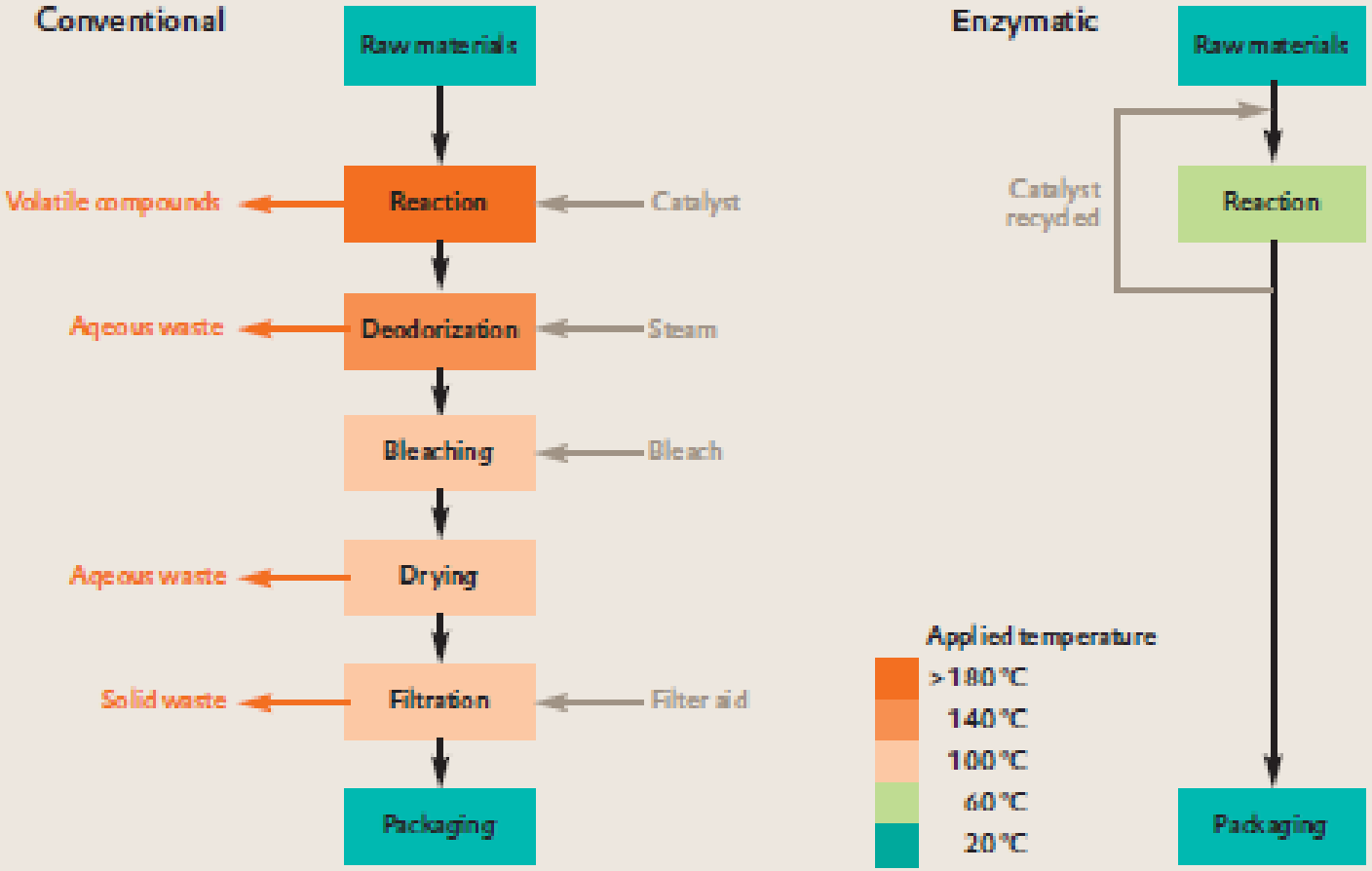


Myristyl miristate: emollient

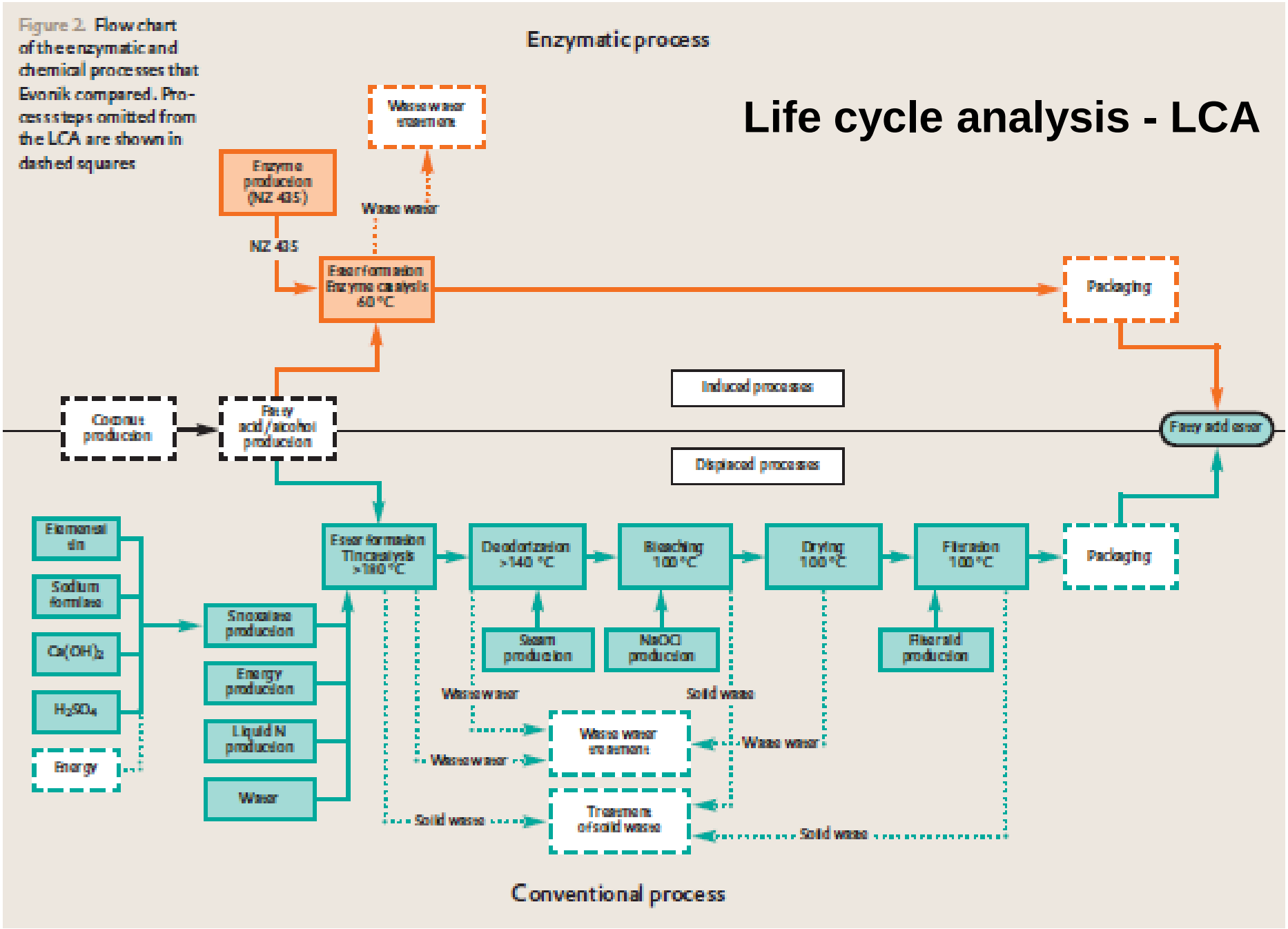
Life cycle analysis - LCA

Figure 1. Using the production of myristyl myristate as their model, Novozymes and Evonik are the first companies to conduct an environmental life cycle assessment for both the enzymatic and chemical manufacture of an emollient ester for cosmetics





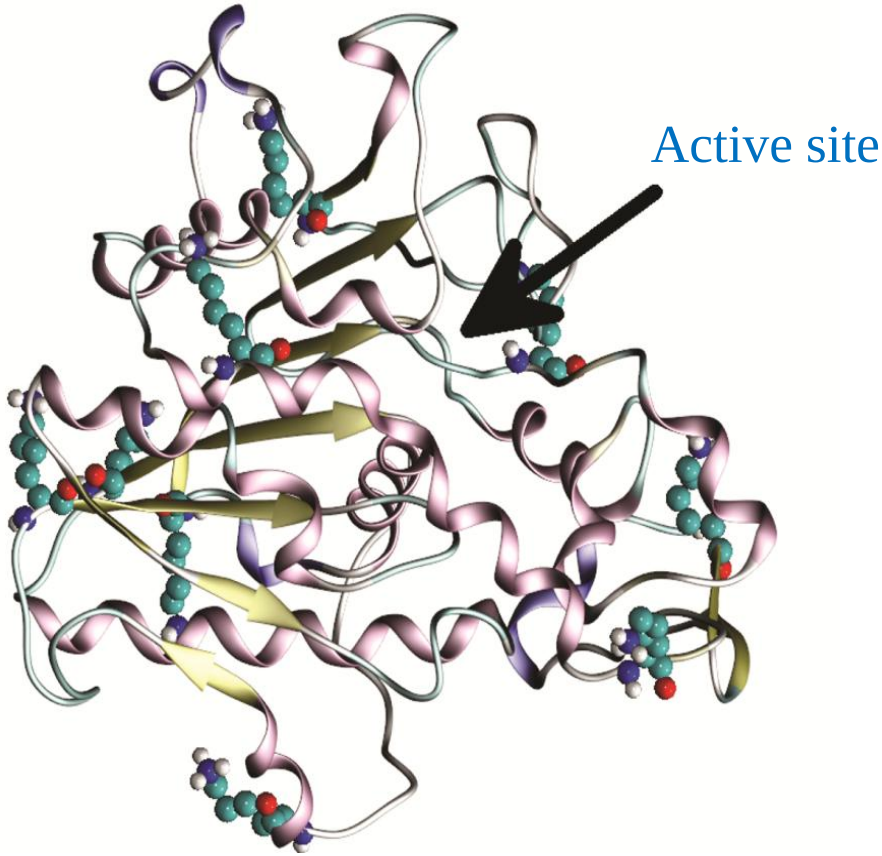
Life cycle analysis - LCA



Results of the life cycle assessment				
5 ton scale				
		Conventional	Enzymatic	Savings %
Energy	GJ	22.5	8.63	62
Global warming	kg CO_2 eq.	1,518	582	62
Acidification	kg SO_2 eq.	10.58	1.31	88
Nutrient enrichment	kg PO_4 eq.	0.86	0.24	74
Smog formation	kg C_2H_4 eq.	0.49	0.12	76

Main contributors to environmental impact					
	Fossil energy %	Global warming %	Acidification %	Nutrient enrichment %	Smog formation %
Tin	15	15	70	55	45
Heating energy	70	70	20	35	40
NaOCl	5	5	5	5	5
Sodium formiate	<1	<1	<1	<1	1
Filter aid	2	<1	<1	5	1

Lipase B from Candida antarctica: the lid is very small. There is no evidenti conformational modification nor interfacial activation in the presence of hydrophobic phases (esterase like enzyme)



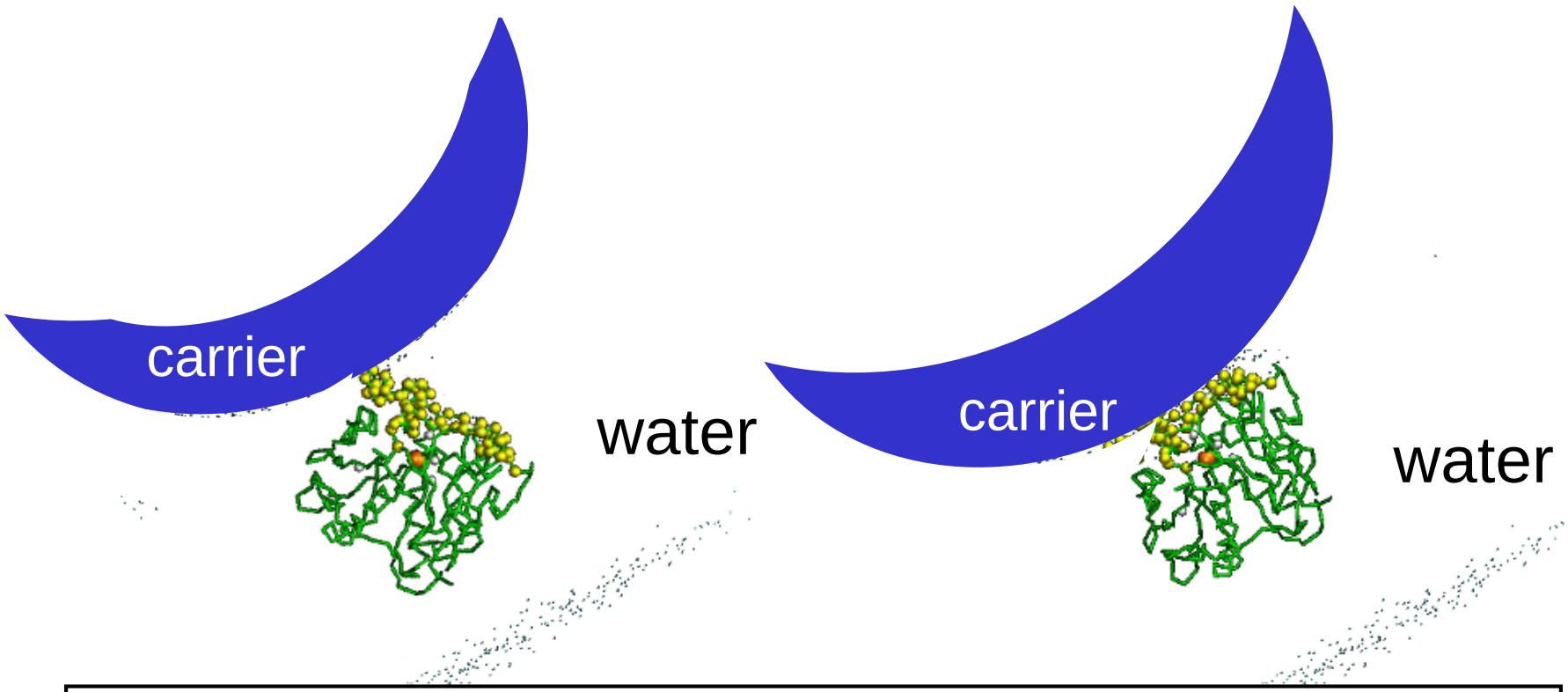
Adv. Synth. Catal. 2011, *353*, 2466 – 2480

Native lipases available on the market

Source	Commercial Name	Supplier
Aspergillus niger lipase	Lipase AS	Amano
Candida antarctica lipase B	Lipozyme CALB L	Novozymes
Candida cylindracea	Lipase OF	Meyto Sangyo Co. Ltd.
Candida lipolytica	-	Amano
Candida rugosa	Lipase AY “Amano” 30	Amano
Candida rugosa	-	Sigma
Candida rugosa	-	Meyto Sangyo Co. Ltd.
Chromobacterium viscosum	-	Asahi Chemical Industry
Geotrichum candidum	Amano GC-4	Amano
Humicola insolens	Lipozyme TL 100L	Novozyme
Papaya latex lipase	Carica papaya latex	Sigma
Penicillium camembertii	Lipase G	Amano
Penicillium expansum	-	Shenzhen Leveking
Penicillium roqueforti	Lipase R “Amano”	Amano
Porcine pancreas Type II	-	Sigma
Pseudomonas cepacia lipase	Lipase PS “Amano” SD	Amano
Pseudomonas fluorescens lipase	*Pseudomonas fluorescens* (AK)	Amano
Rhizomucor miehei	Palatase 20000	Novozyme
Rhizopus delemar	-	Seikagaku Kogyo Co.
Rhizopus japonicus	Lipase A-10FG	Nagase Biochem., Ltd.
Rhizopus niveus	Newlase F	Amano
Rhizopus oryzae	Amano F-AP15	Amano
Thermomyces lanauginosus	Lipolase 100L	Novozymes

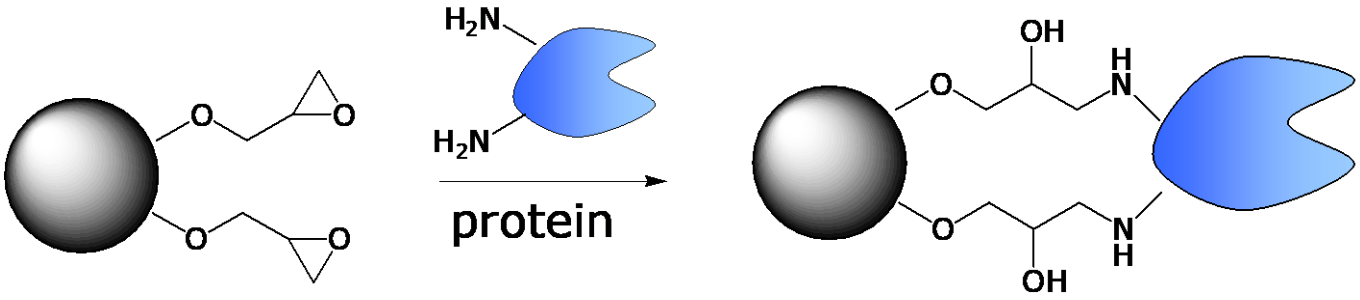
Some examples of immobilized lipases reported in the literature

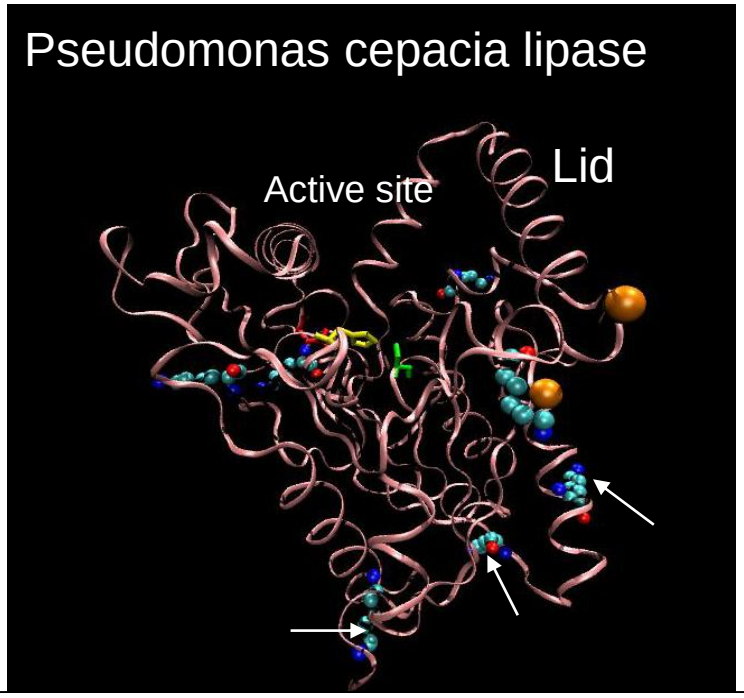
Source	Product and Supplier	Method	Support
C. antarctica	Novozym®435 Sprin-CALB Lipobond	Adsorption	Acrylic resin
Porcine pancreas	- (Sigma)	Ionic interaction	Ionic resin
P. cepacia	Lipase PS IM (Amano)	Deposition	Celite
P. cepacia	Lipase PS-C (Amano)	Deposition	Ceramic
R. miehei	Lipozyme® RM-IM (Novozymes)	Ionic interaction	Duolite A568
T.lanuginosus	Lipozyme®TL IM (Novozymes)	Granulate with binder	Silica

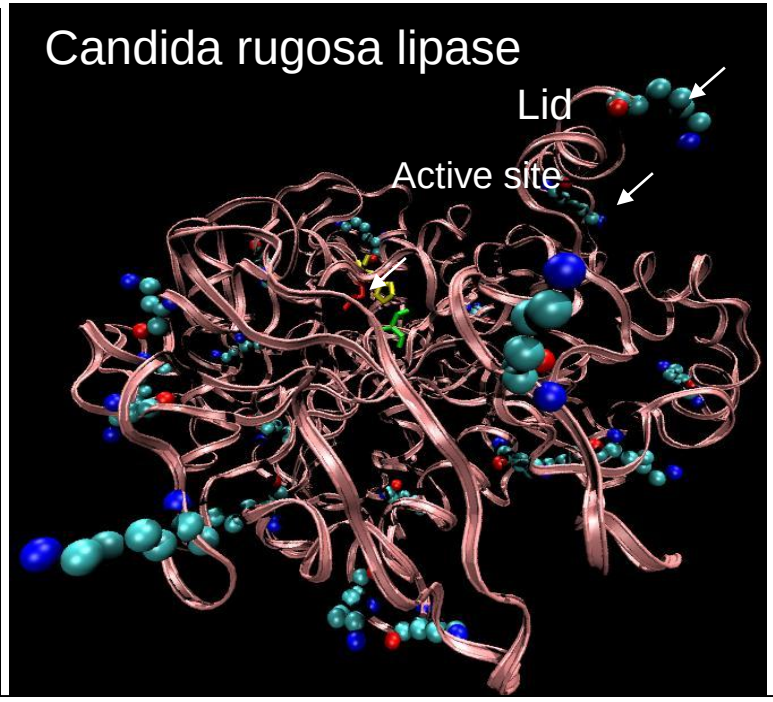


Immobilization of lipases on organic hydrophobic resins + aqueous buffer: the active site will point towards the carrier. Wrong orientation!

Structural comparison of lipases: Lys on surface





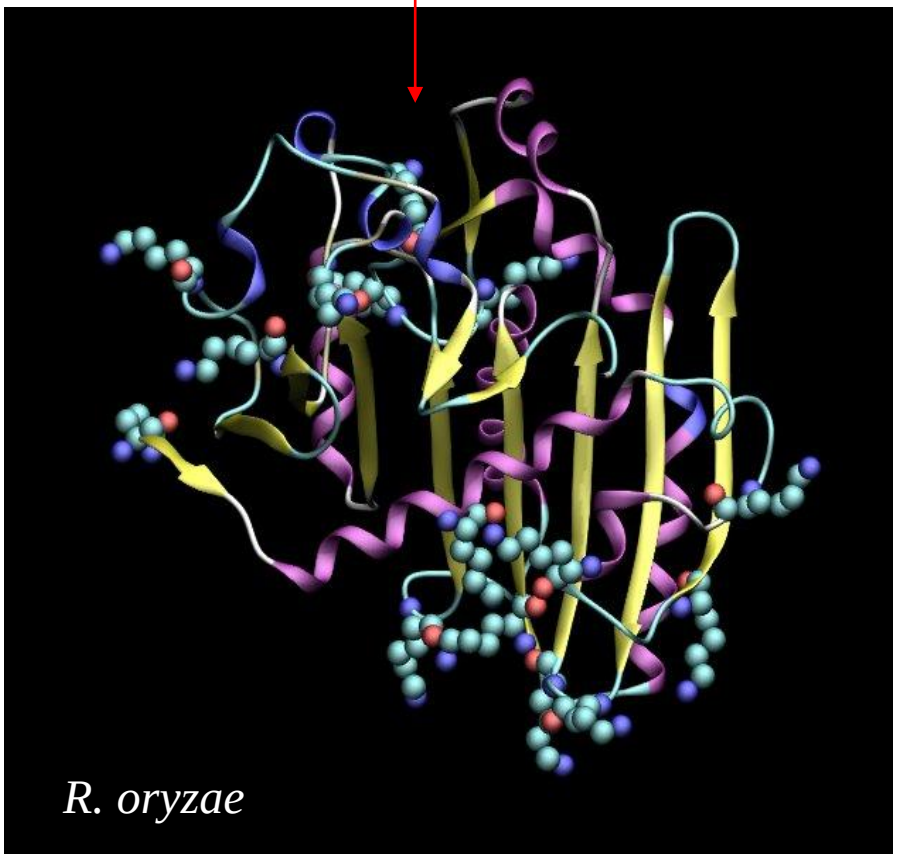


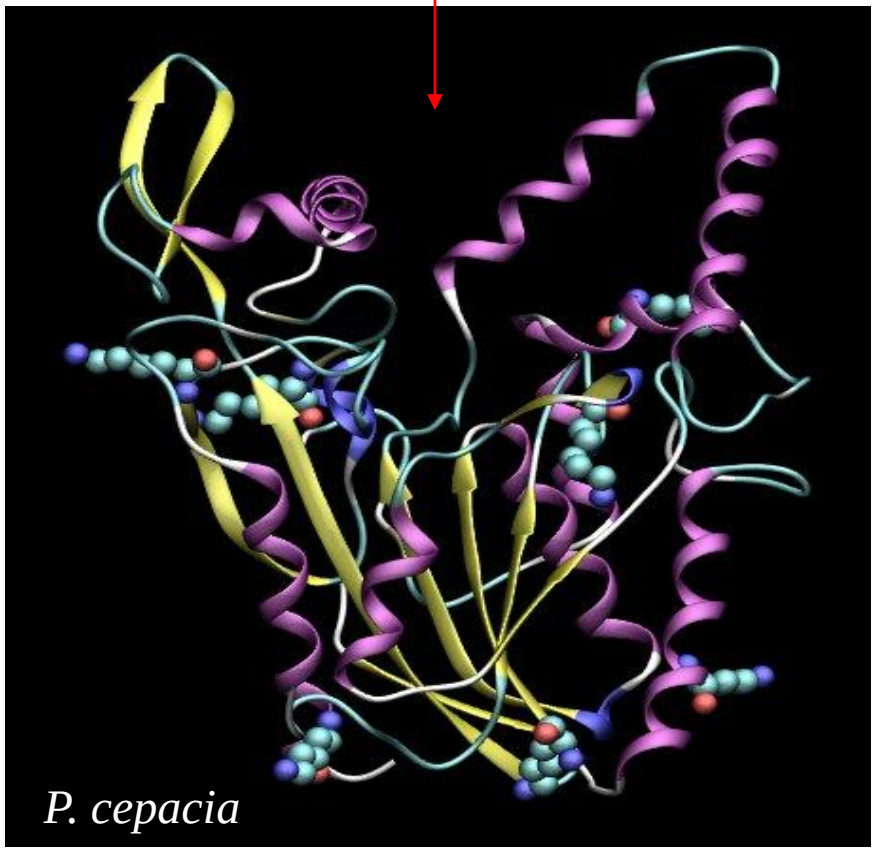
Lys residues on the surface

Glycosylation sites (theoretical)

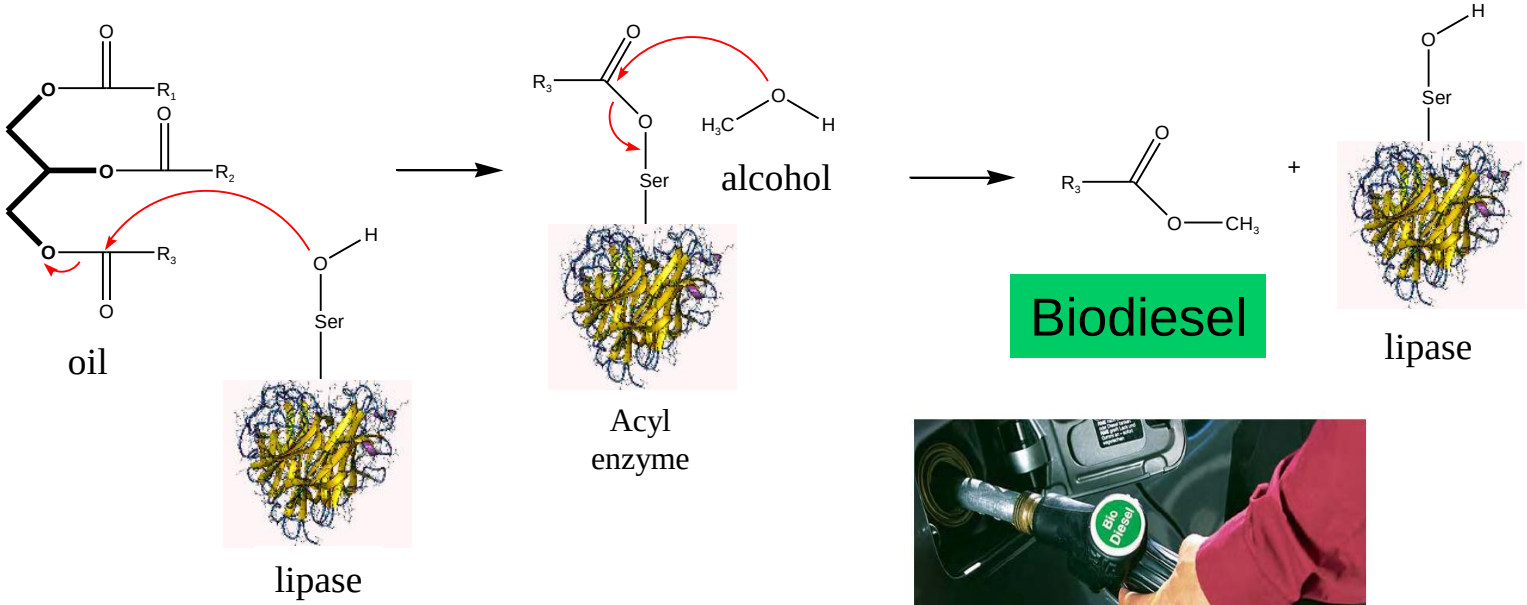
Arrows indicate crucial Lys residues on the surface (space-filling representation)

Structural comparison of lipases: Lys on surface





Enzymatic synthesis of biodiesel: advantages



Demonstration plants only

a) possibility of using waste oils and oils containing fatty acids
b) use of steichiometric amounts of the alcohols
c) reduction of wastes
d) mild and neutral reaction conditions
e) higher purity of the glycerol obtained as secondary product

Lipases in food industry

Specialty esters

-Structured triglycerides containing long chain fatty acids are an important class of raw materials for emulsions since their sensory properties vary with the fatty acid composition (content, position and saturation degree of fatty acids).

Replacement of a fatty acid with a different one to confer different properties

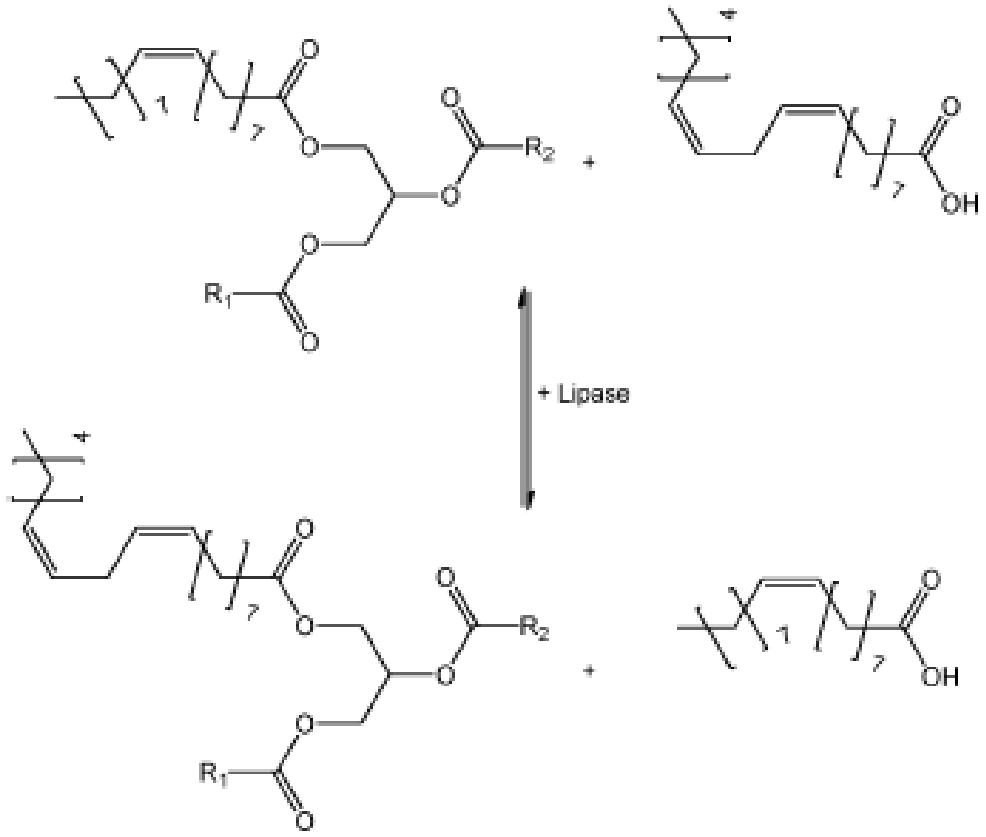


Fig. 2 Representative reaction scheme for the preparation of structured triglycerides. Here, an oleic acid moiety is replaced by a linoleic acid moiety.

Lipases in the synthesis of structured esters

The possibility of using lipases in low-water environments has made possible their application for the production of structured triacylglycerols by enzymatic inter- and transesterifications.

This mild and selective approach has been utilized for producing

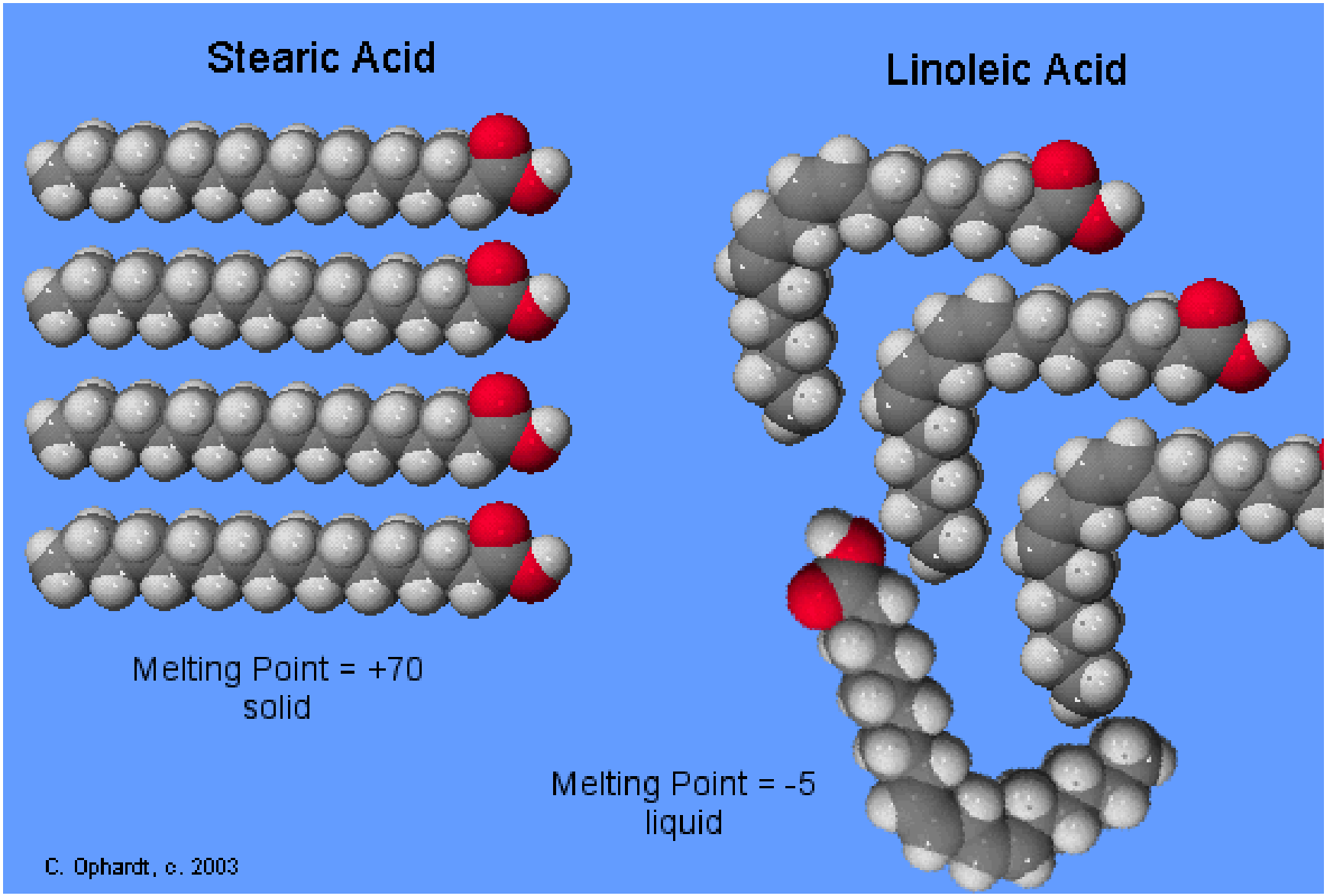
- **cocoa-butter equivalent**
- **infant-formula substitutes.**
- **triacylglycerols containing polyunsaturated fatty acids (PUFA)**

Synthesis of cocoa butter analogues

Cocoa butter has a melting point close to 37°C.
It is mainly composed of SOP and SOS triacylglycerols (where S = stearic and O = oleic, P = palmitic).

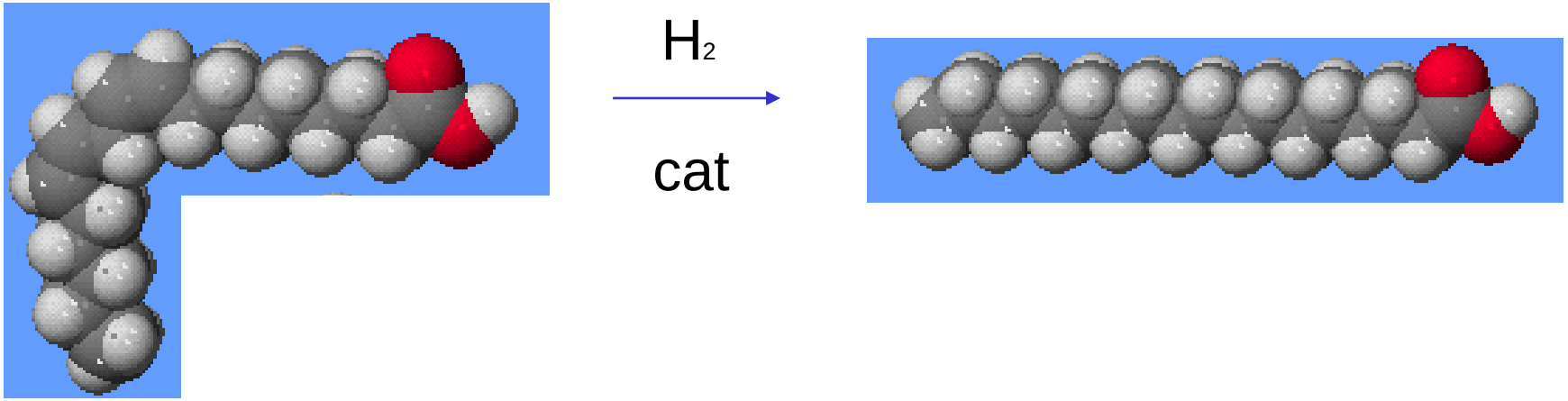
Palm oil mid-fraction is liquid at room temperature an rich of POP triacylglycerol (where P = palmitic acid) and can be converted into cocoa butter by chemo- and regiospecific lipases able to introduce stearic acid in position sn_1 and sn_3. The biotransformation can be carried out in solvent-free medium, where palm oil is used in large excess.

Unsaturated fatty acids have lower melting point

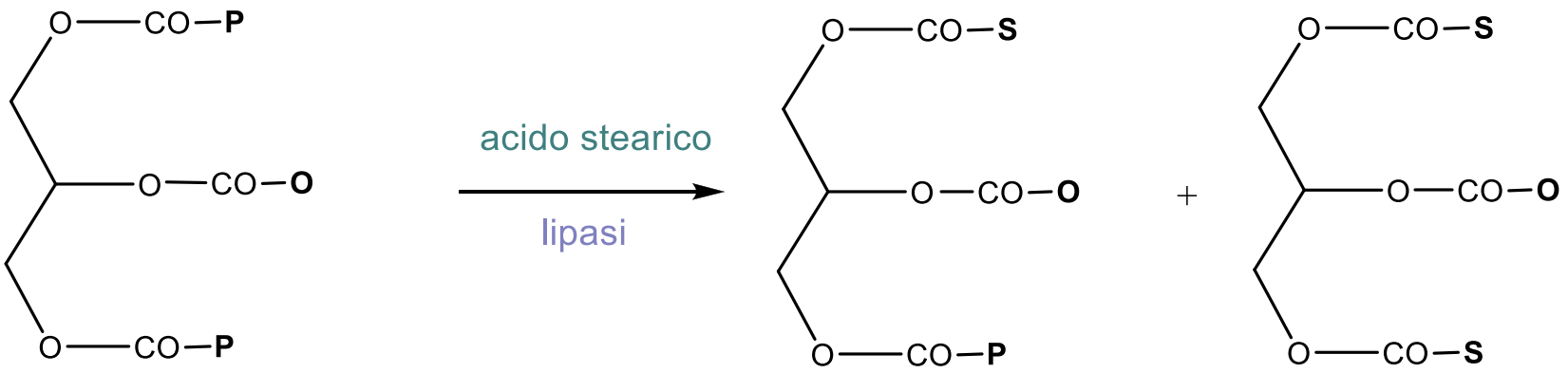


The chemical alternative: hydrogenated fatty acids

Hydrogen and a metal catalyst operating at about 100°C and under vacuum.
Unsaturated fatty acids are converted in saturated fatty acids



Transesterification catalyzed by lipase from Mucor mihei



Palm oil

Cocoa butter analogue

P: palmitic – saturated 16Carbon atoms
O: oleic – unsaturated
S: stearic – saturated 18 Carbon atoms

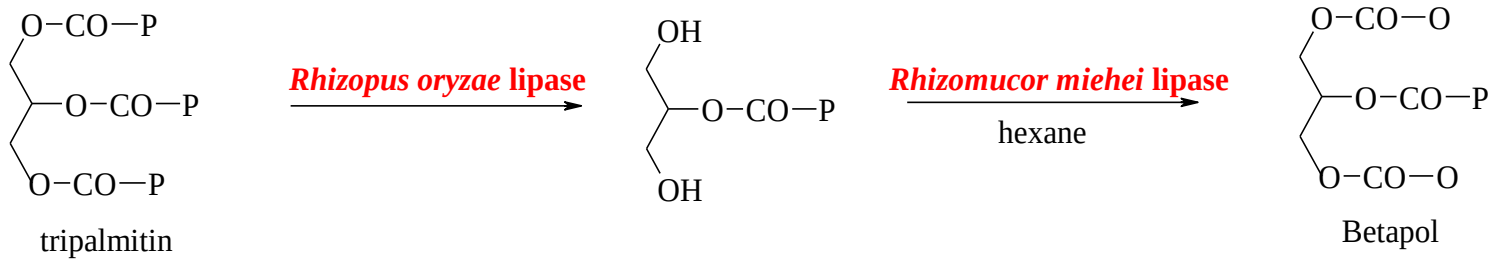
Table 3. Important commercially available lipases.

Origin	Code[a]	*M* [kDa] (rounded)	Specificity (remarks)	Applications
of mammalian origin				
human pancreatic lipase	HPL	50	*sn*-1,3	
human gastric lipase	HGL	50	*sn*-3 (acid-stable)	
porcine pancreatic lipase	PPL	50	*sn*-1,3	organic synthesis, digestive aid
guinea pig pancreatic lipase	GPL-RP2	48	*sn*-1,3 (phospholipase A1 activity)	
of fungal origin				
Candida rugosa	CRL	60	nonspecific	organic synthesis
Candida antarctica B	CAL	60	*sn*-1,3	organic synthesis
Geotrichum candidum	GCL	60	cis-Δ^9 (unsaturated fatty acids)	oleochemistry
Humicola lanuginosa	HLL	30	nonspecific	detergents
Rhizomucor miehei	RML	30	*sn*-1,3	cheese manufacturing
Aspergillus oryzae	AOL			cheese manufacturing
Penicillium camembertii	PEL	30	*sn*-1,3	monoglycerides
Rhizopus delemar	RDL	41	*sn*-1,3 (phospholipase A1 activity)	oleochemistry
Rhizopus oryzae	ROL	41	*sn*-1,3 (phospholipase A1 activity)	oleochemistry
Rhizopus arrhizus	RAL	41	*sn*-1,3 (phospholipase A1 activity)	oleochemistry
of bacterial origin				
Pseudomonas glumae	PGL	33	nonspecific	detergent enzyme, organic synthesis
Burkholderia cepacia	PCL/BCL	33	nonspecific	organic synthesis
Pseudomonas pseudoalcaligenes	PPL	33	*sn*-1,3	detergents
Pseudomonas mendocina	PML	33	*sn*-1,3	detergents
Chromobacterium viscosum	CVL	33	*sn*-1,3	organic synthesis
Bacillus thermocatenulatus	BTL-2	43	*sn*-1,3 (thermoalkalophilic)	
Fusarium solani (hydrolyzes cutin)	FSL	22		detergents

[a] Other appreviations for lipases used in this article: PSL (*Pseudomonas species* lipase), PFL (*Pseudomonas flourescens* lipase), HLL (human lipoprotein lipase), LPL (lipoprotein lipase). Lipases can be obtained commercially from many suppliers. Important original producers are Novo-Nordisk (Baegsvard, Denmark), Genencor International B. V. (Delft, The Netherlands), Boehringer-Mannheim (Mannheim, Germany), and Amano Co. (Nagoya, Japan).

The observation that lipases selectively catalyze both hydrolysis and esterifications has been further exploited for the obtainment of Betapol® from tripalmitin (palm oil). Betapol® consists of triglyceride fatty acids commonly found in vegetable and animal fats. A similarity to human milk fat indicated a potential use in **infant formulae** as well as for food use in general.

Tripalmitin is firstly hydrolysed with a lipase from Rhizopus oryzae able to remove only the fatty acids in position 1 and 3. The resulting 2-mono-palmitin has been esterified with oleic acid using the Rhizomucor miehei lipase in organic solvent



P: palmitic – saturated 16Carbon atoms
O: oleic – unsaturated

The biotransformation at industrial level

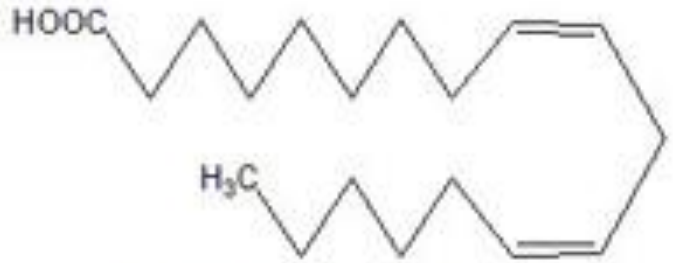
The lipase is immobilized onto porous silica granulates which are insoluble in oil. (see immobilized enzymes)

The immobilized lipase Lipozyme® TL IM viewed through a light microscope. The enzyme is bound to a silica carrier

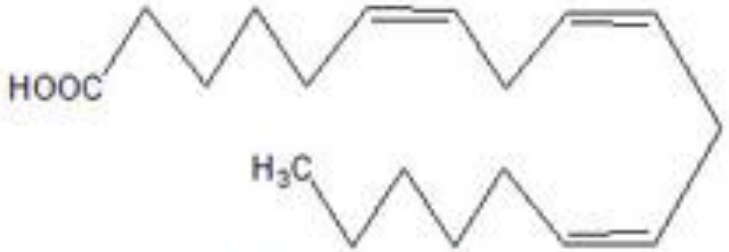
Lipases have been extensively used for the hydrolysis and transesterification of triacylglycerols. Chemo- and regio-selective hydrolysis of triacylglycerols has been exploited for enrichment of specific fatty acids, such as **polyunsaturated fatty acids (PUFA) from fish oils**.

PUFAs are increasingly used as food additives, pharmaceuticals and nutraceuticals because of their metabolic benefits. Many PUFAs are essential for normal synthesis of lipid membranes and prostaglandins.

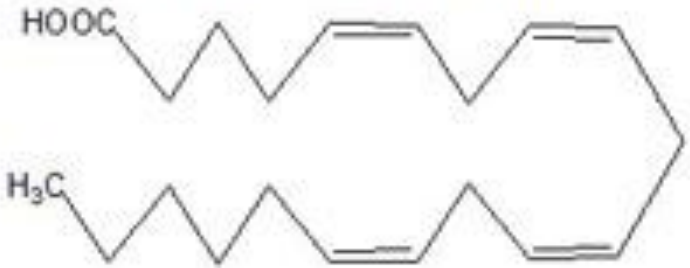
Omega-6 Fatty Acids



Linoleic Acid (18:2n-6)

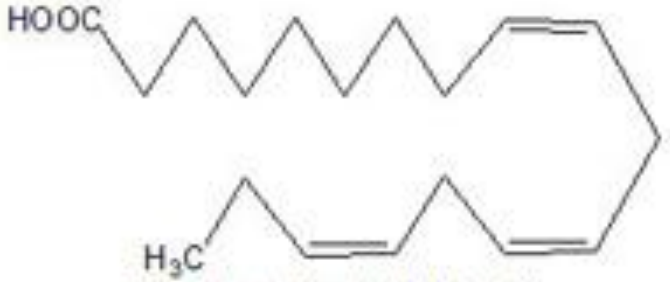


γ-Linolenic Acid (18:3n-6)

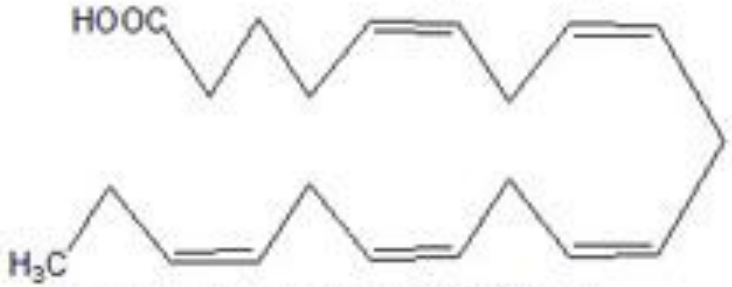


Arachidonic Acid (20:4n-6)

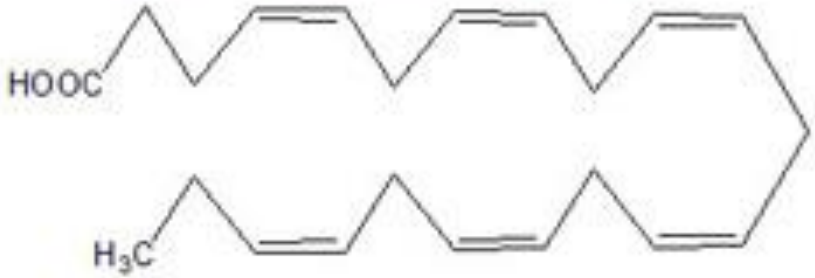
Omega-3 Fatty Acids



α-Linolenic Acid (18:3n-3)

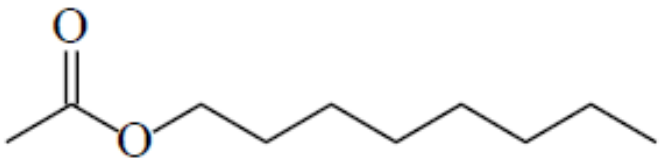


Eicosapentaenoic Acid (20:5n-3)



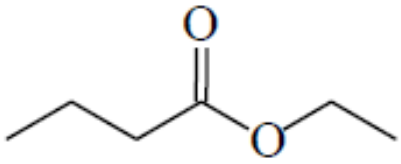
Docosahexaenoic Acid (22:6n-3)

Esters synthesised by lipases used as aromas in food and pharma sector



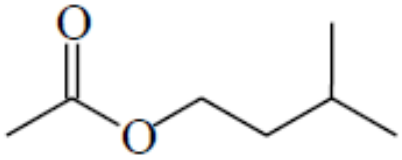
ottil etanoato
(ottil acetato)

arancia



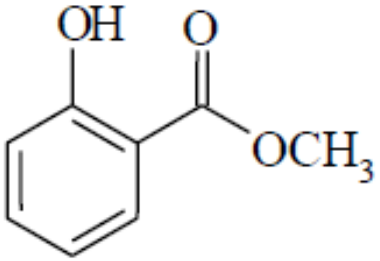
etil butanoato
(etil butirrato)

ananas



3-metilbutil etanoato
(isopentil acetato, isoamil acetato)

banana



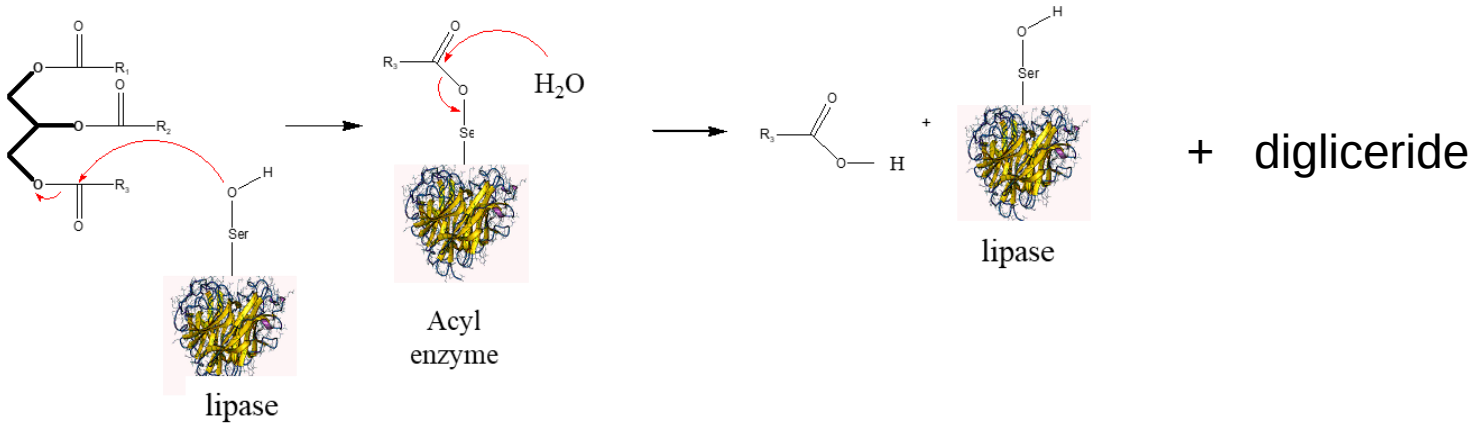
metil 2-idrossibenzoato
(metil salicilato)

menta

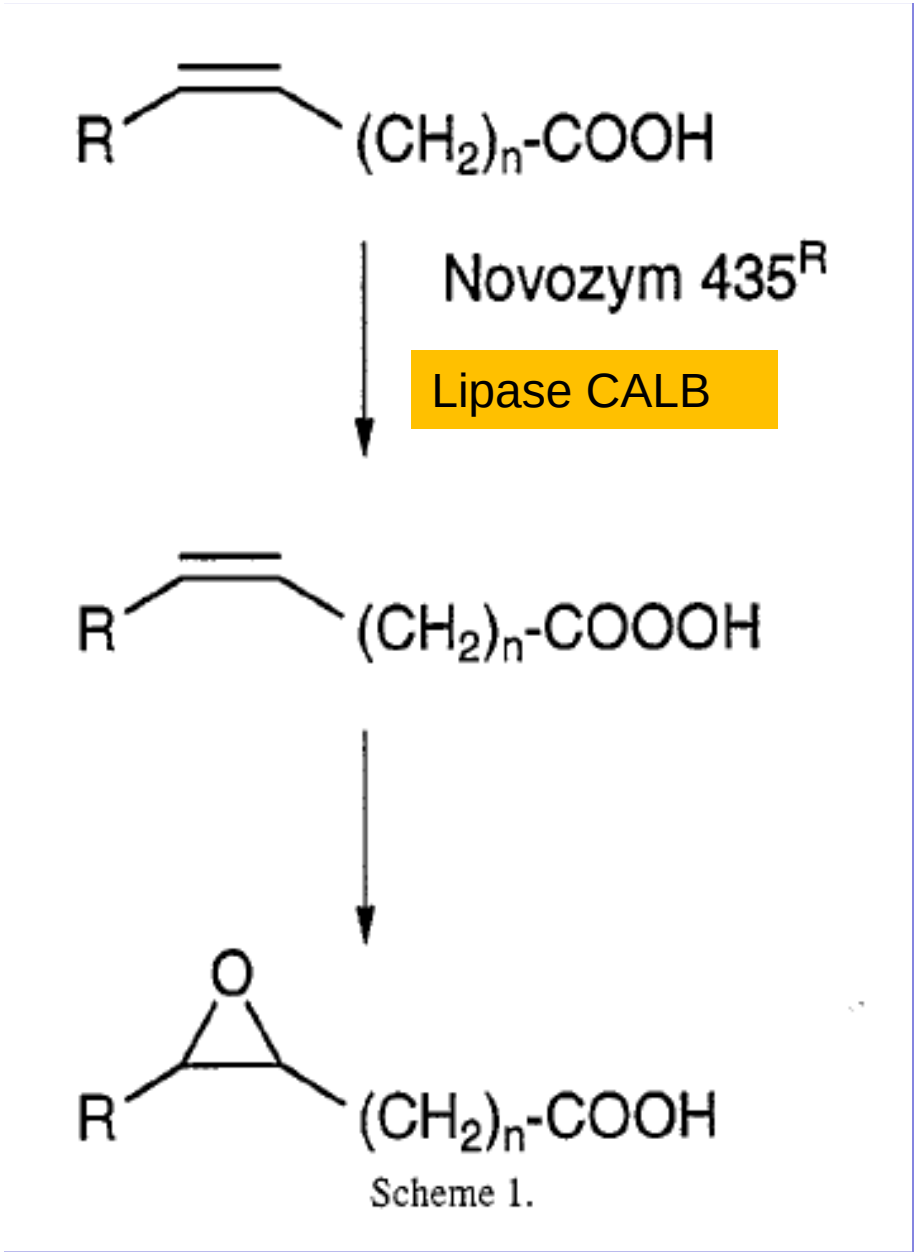
The International Organization of Flavor Industries defines the **natural flavoring** ingredients such as **"those obtained through appropriate physical, enzymatic or microbiological transformations from a material of plant or animal origin, in the natural state or prior transformation for human consumption**". Therefore compounds obtained from the synthesis of natural, biological substrates are considered as natural, while the same chemically produced materials are not. The production of a variety of products from natural processes takes on great significance for the final consumer who attributes to the "natural" product greater safety and genuineness, whether they are food consumption products or cosmetic products (for example creams). These products substantially determine price increases. Thus, the manufacture of various products by means of lipase catalysis turns out to be profitable for the industry

Partial hydrolysis of triglyceride to obtain emulsifiers

The partial hydrolysis of triglycerides to increase the monoglyceride content is obtained by adding lipases to the bread dough: this leads to a delay of the refining.

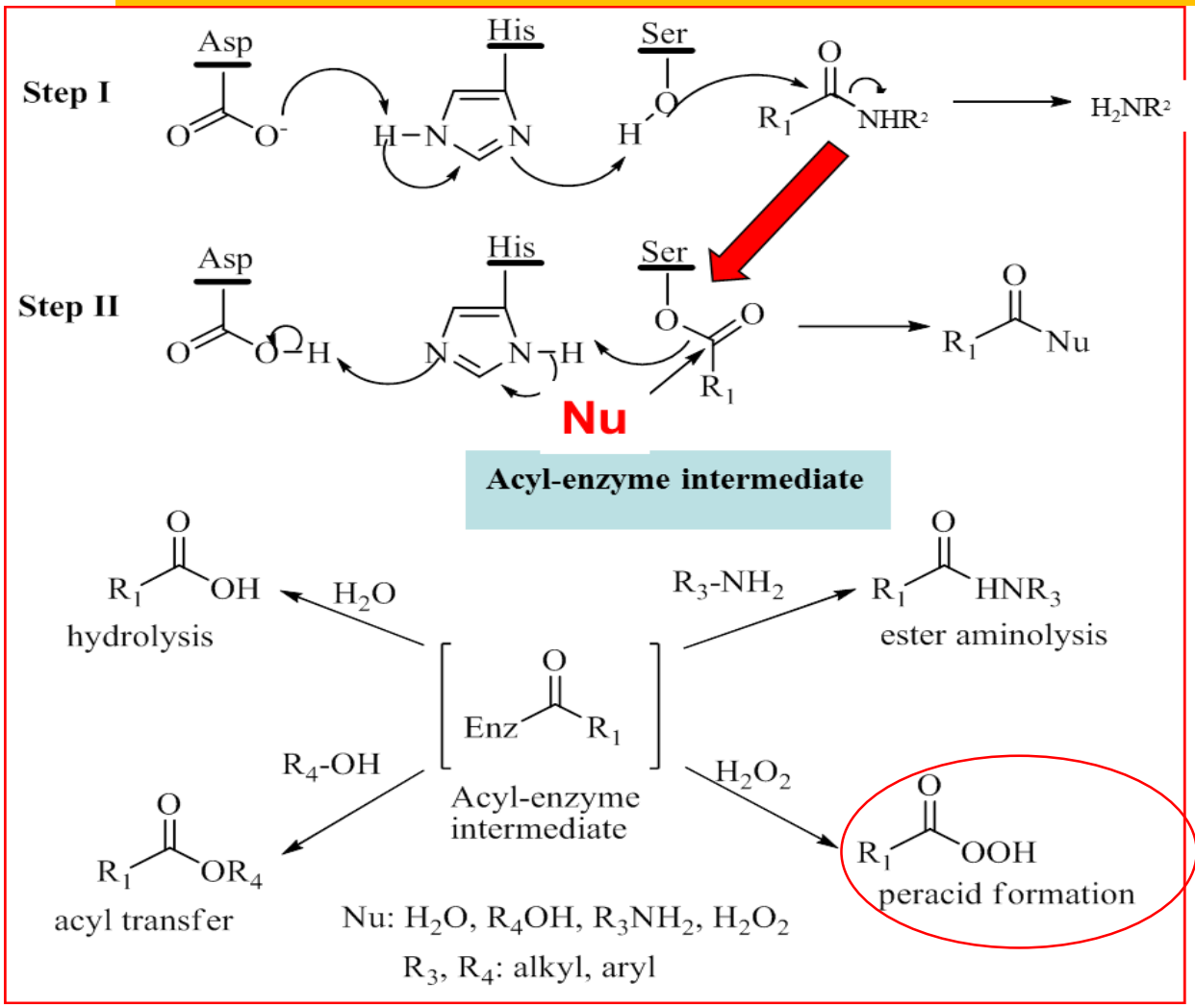


Chemoenzymatic epoxidation of C=C bonds of thiglycerides and unsaturated fatty acids



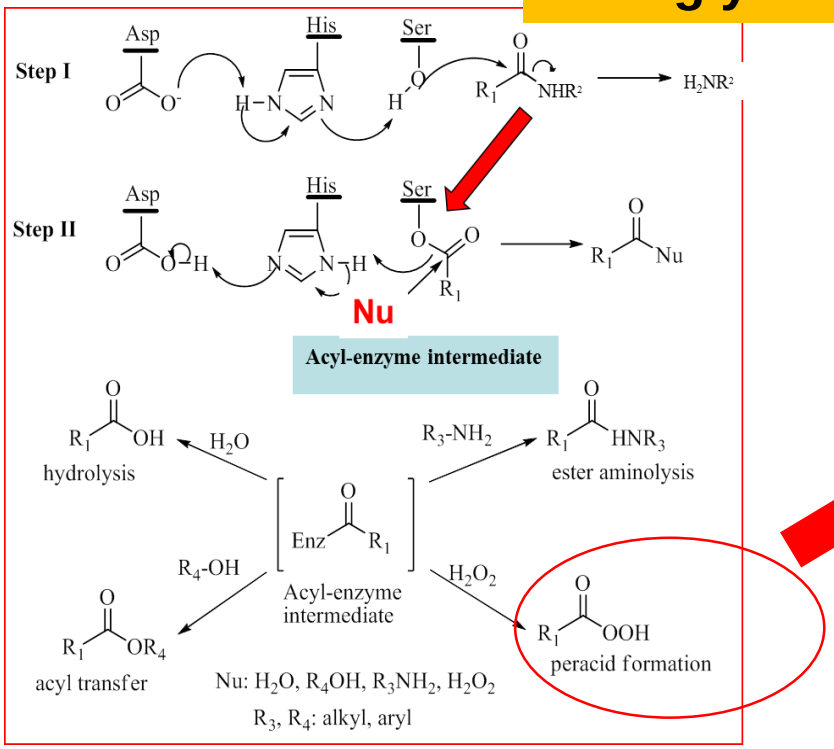
Scheme 1.

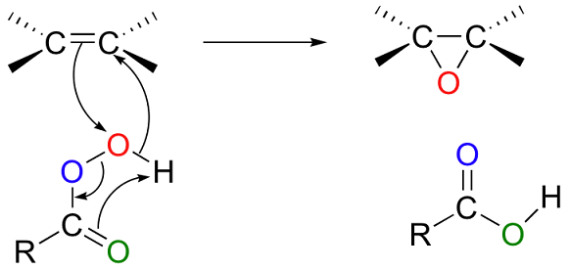
Chemoenzymatic epoxidation of C=C bonds of thiglycerides and unsaturated fatty acids



1.Enzimatic formation of perossiacid

Chemoenzymatic epoxidation of C=C bonds of thiglycerides and unsaturated fatty acids





2.Chemical epoxidation

The obtained epoxidized oils are used as bio-lubricants or bio-plasticizers.

Enzymatic synthesis of bio-based oil derived biolubricants, plasticizers and poliesters

polymers

MDPI

Article

Understanding Marine Biodegradation of Bio-Based Oligoesters and Plasticizers

Federico Zappaterra [1,†], Monia Renzi [2], Manuela Piccardo [2], Mariachiara Spennato [1], Fioretta Asaro [1], Martino Di Serio [3], Rosa Vitiello [3], Rosa Turco [3,4], Anamaria Todea [1,*] and Lucia Gardossi [1]

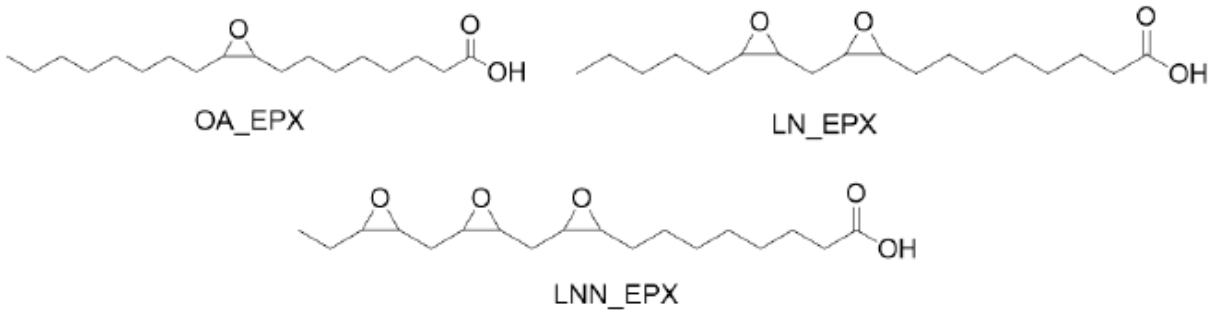


Figure 4. Structures of the products obtained from the enzymatically catalyzed epoxidation of oleic, linoleic, and linolenic acids. Codes refer to marine biodegradation tests.

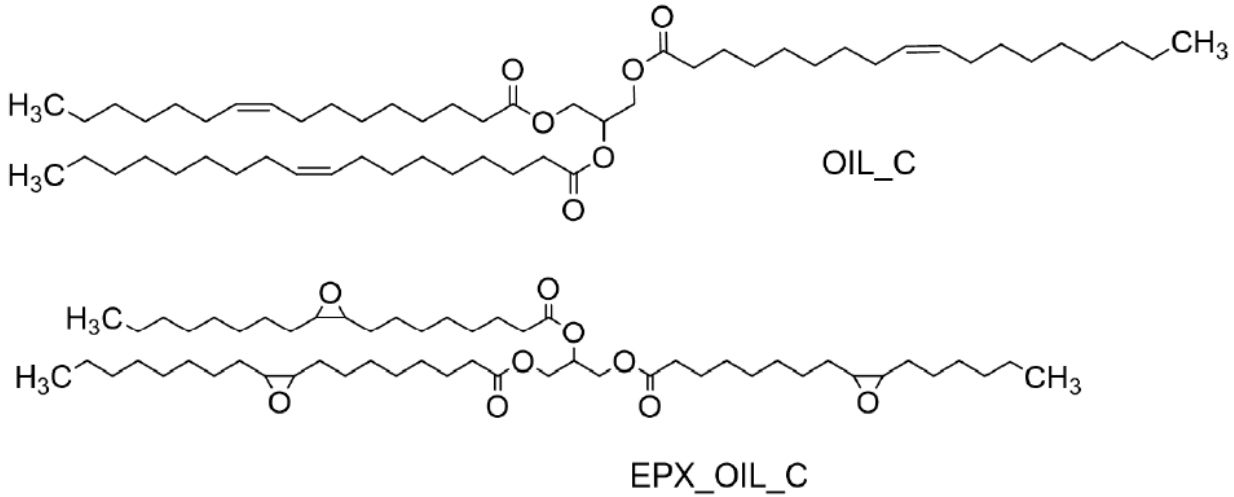


Figure 2. A schematic representation of the structure of an unsaturated triglyceride (triolein) and the corresponding epoxidized products. The cardoon seed oil is actually composed of a variety of saturated and unsaturated fatty acids, as reported above. The figure aims at illustrating the transformation of a triglyceride in a simplified way. Codes refer to marine biodegradation tests.

Lipases for the enzymatic synthesis of bio-based poliesters

The process: enzymatic solvent-free polycondensation of bio-based monomers

Advantages:

- **Selective catalysis** *(OK for multifunctional monomers)*
- **Mild reaction conditions (~70°C)** *(OK for labile monomers)*
- **Control of molecular weight and structure** *(Low molecular weight)*

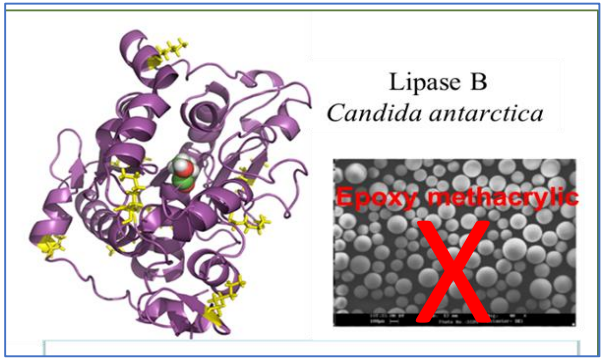
M_n <2000 g/mol when using recyclable enzymes in solvent-free systems

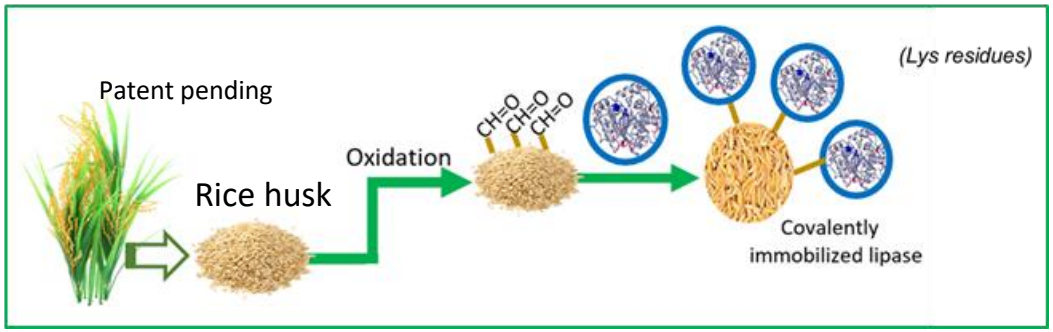
- **Solvent-free reactions feasible**
- **Enzyme recyclability**

Lipase B
Candida antarctica

A. Guarneri et al. Adv. Synth. Cat., 2019, 361, 2559-2573

Optimization of solvent-free enzymatic polycondensation: immobilization of the enzyme





Product	Allowable cost contribution of enzyme (€/kg)	Range of required productivity for immobilized enzymes
Pharma	10	50-100 kg product / kg biocatalyst
Bulk chemical	0.05	2000-10000-kg product / kg biocatalyst

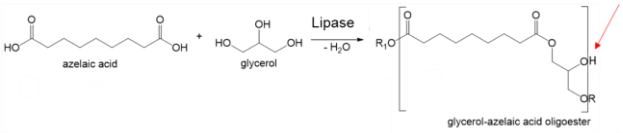
- **Robust, cheap, immobilized enzymes**

  Prevent enzyme leaching

  Enable recycling

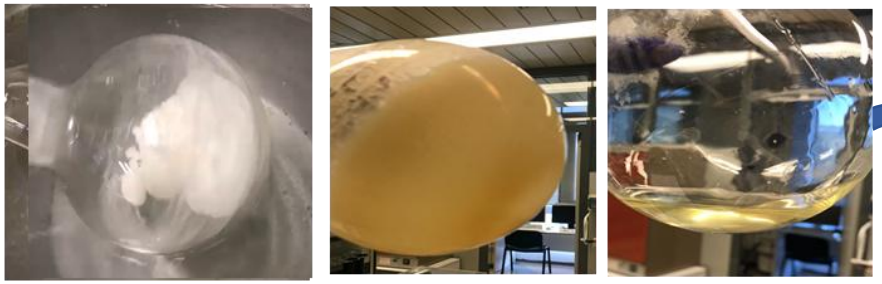
Biocatalyst must be recycled efficiently!

Scalable?



Solvent-free

Synthesis of short oligomers

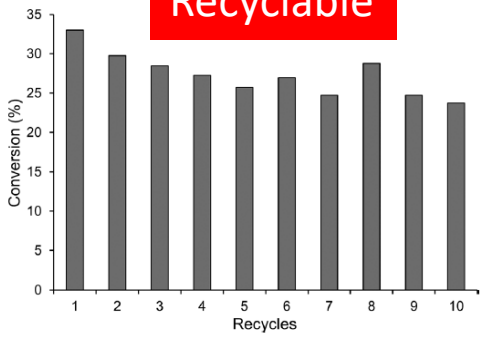


- **High viscosity**
- **No mechanical/magnetic stirring**

  Mechanical damage of carrier prevented
- **Thin film**

  Optimal mass and heat transfer, carrier preserved

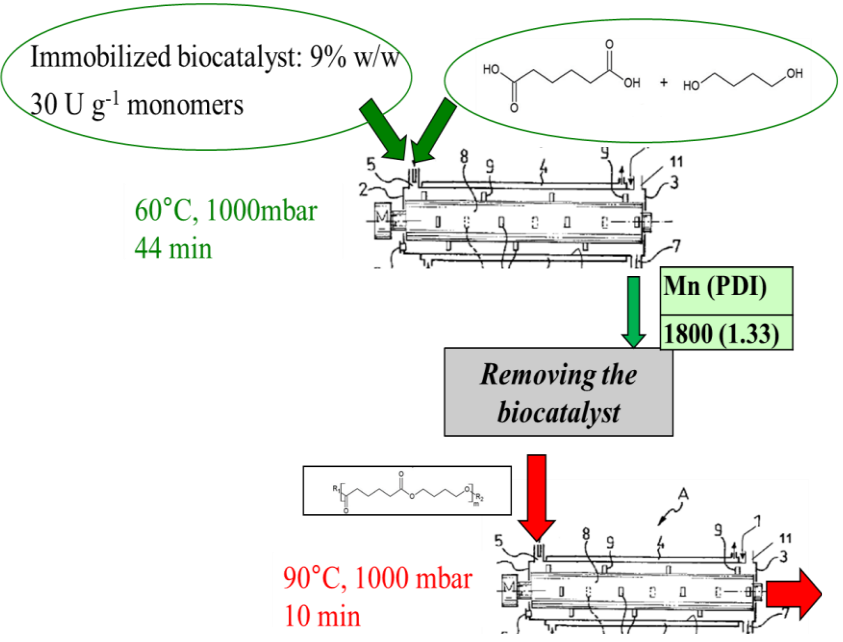
Recyclable

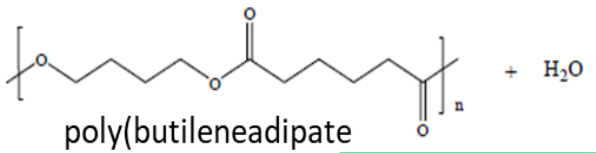


ChemSusChem, 2022, 15 (9), e202102657

Two step polycondensation in turbo-reactor: catalyst-free elongation

Scalable: thin film in turbo-reactor





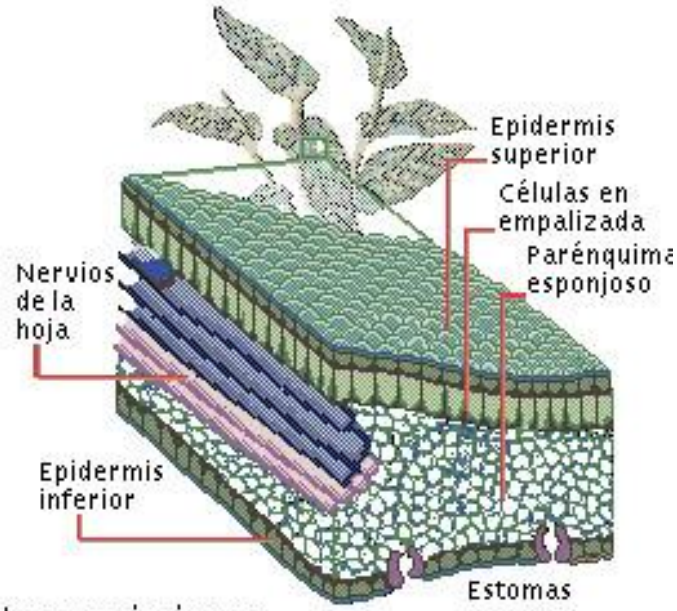
M_n 2900 Da

Patent EP 2 620 462 A1.

Cutinases as alternatives to lipases

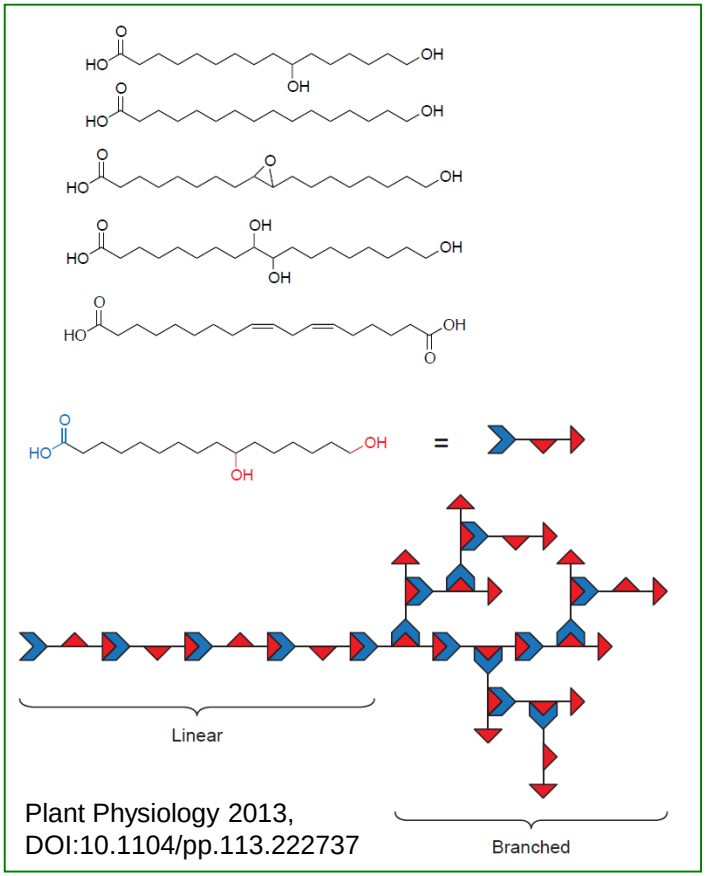
Cutin

The cutin is the hydrophobic waxy substance present in greater quantity inside the protective cuticle that covers the outer parts of the plant tegumentary tissues preventing their drying out.

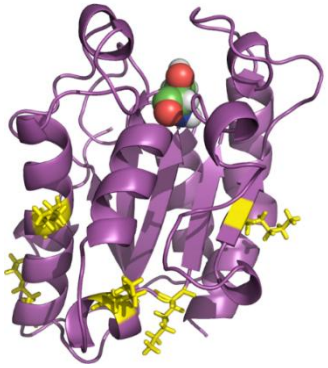


UNIVERSITÀ DEGLI STUDI DI TRIESTE
Dipartimento di
Scienze Chimiche e Farmaceutiche

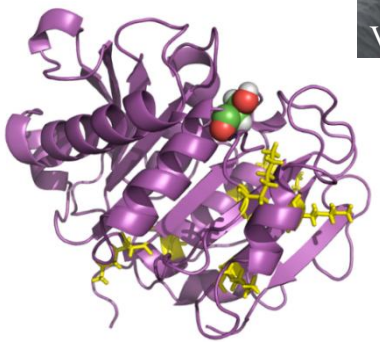
Cutinases are biosynthesized by pathogenic fungi to hydrolyze plant cutin



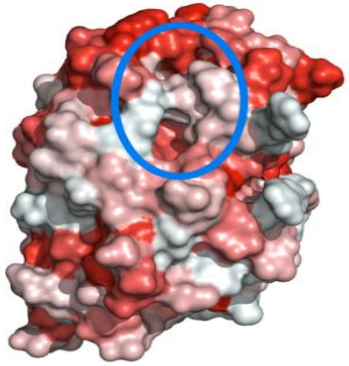
Plant Physiology 2013,
DOI:10.1104/pp.113.222737

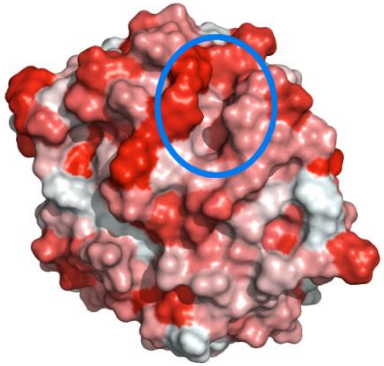


Cutinase
Humicola insolence



Cutinase
Thermob. cellulosilytica

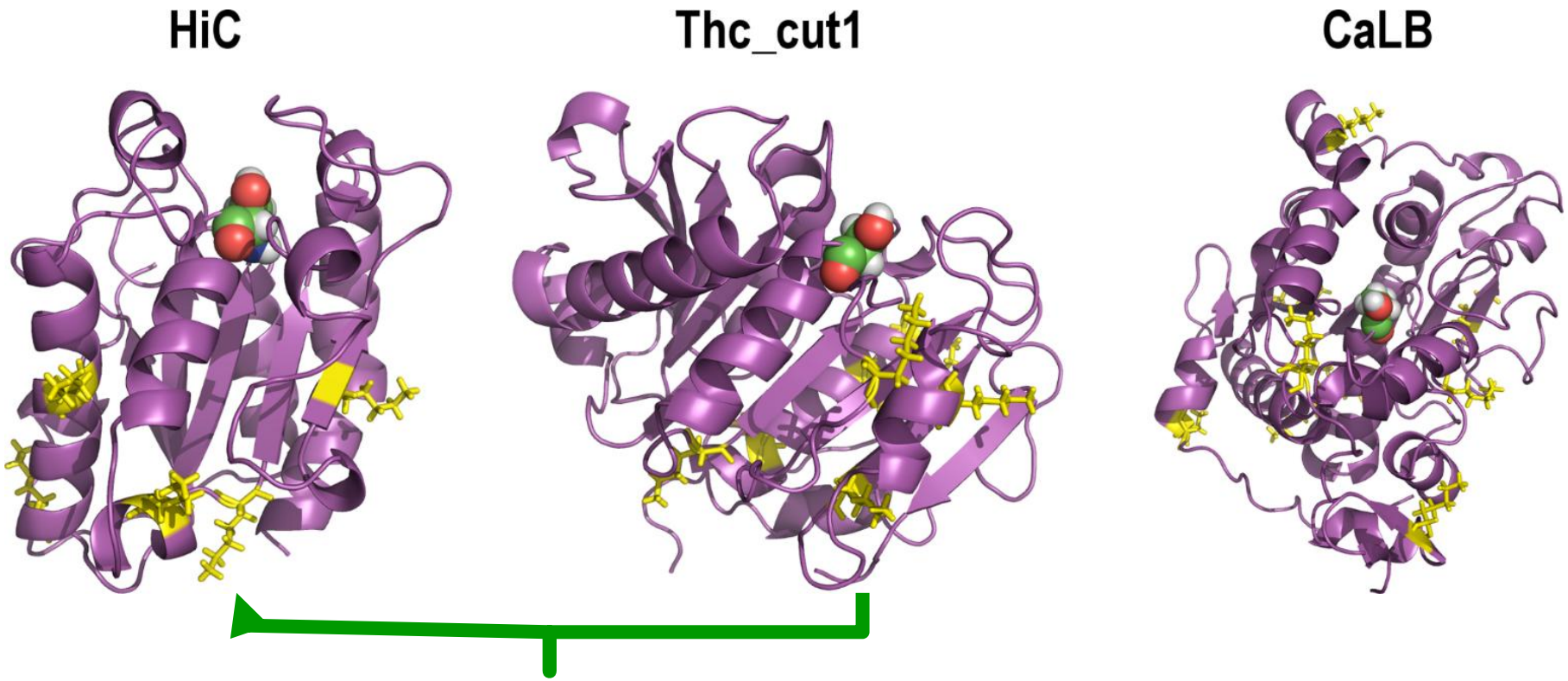




Hydrophobicity

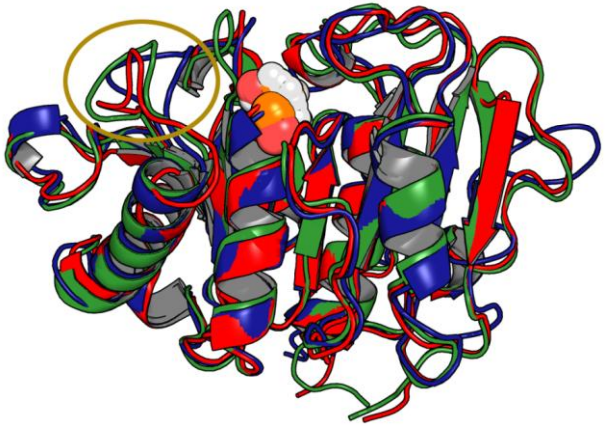
Pellis A. et al. *Catalysts* 2016, *6*, 205.
Pellis, A. et al. *Catal. Sci. Technol.*, 2016, *6*, 3430.

Comparison: cutinases and lipase from *Candida antarctica* (CALB)

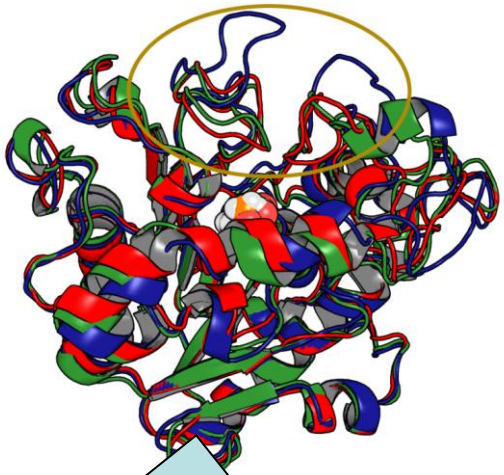


Cutinase *vs* lipase: MD simulations

Thermobifida cellulosilytica
Cutinase 1



CaLB



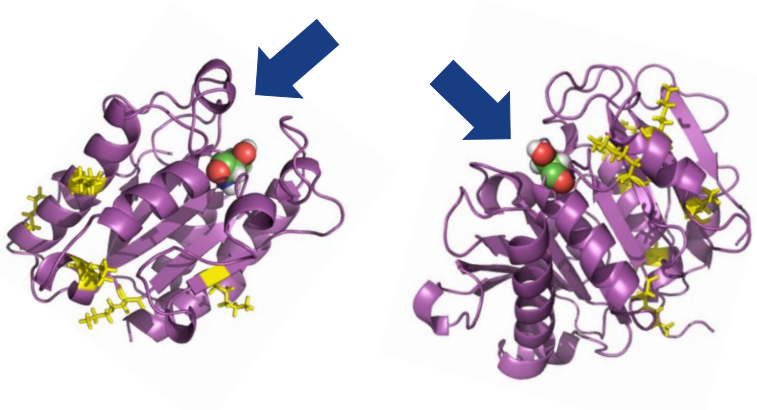
Very bad!!

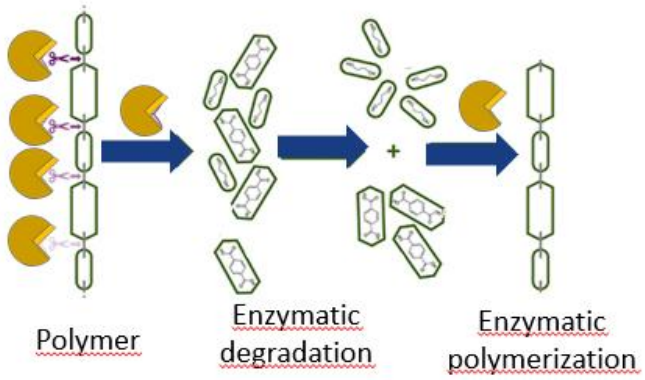
	T	p
70°C; 1000mBar:	+	+
70°C; 70 mBar:	+	-
27°C; 1000mBar	-	+

More accessible

Green Chem., 2017, 2017,**19**, 490-502

Cutinases: enzymes for polymer degradation and re-synthesis





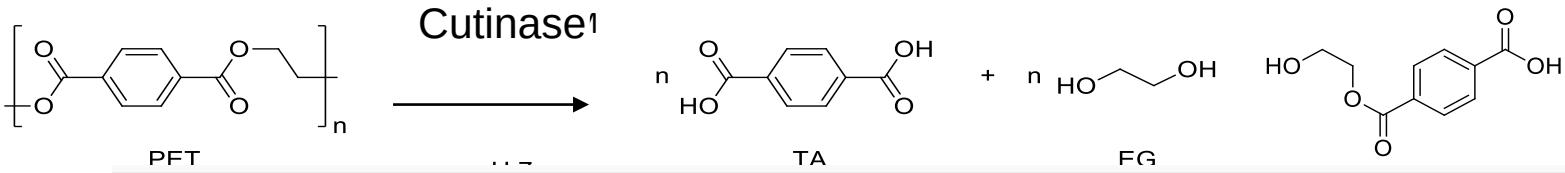
Cutninases: Enzymes produced in nature by pathogenic fungi, able to degrade cutin, a bulky hydrophobic polyester

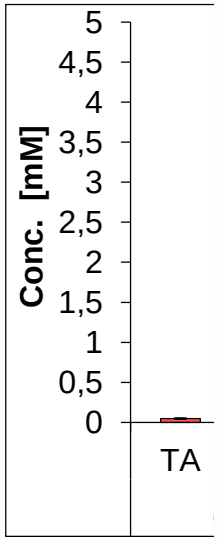
Lucia Gardossi

Pellis A. et al *Green Chem.*, **2017**, *19*, 490-502.

Pellis A. et al. *Catalysts* **2016, *6*,** 205.

Hydrolysis of PET catalyzed by Cutinase 1 from *Thermobifida cellulosilytica*





Menu Cerca Notifiche **la Repubblica** ABBONATI Accedi

Alessandro Pellis: "Ecco la mia plastica riciclabile all'infinito"

di Paola Arosio

▲ Alessandro Pellis

Il giovane ricercatore dell'Università di Genova ha ottenuto un finanziamento europeo per sviluppare polimeri 100% rinnovabili, prodotti attraverso processi sostenibili

Nicola Piovesan, 2016, Thesis, Master in Chemistry, Univ. Trieste

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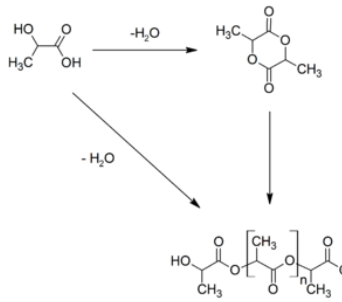
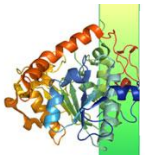
Enzyme-catalyzed functionalization of poly(L-lactic acid)

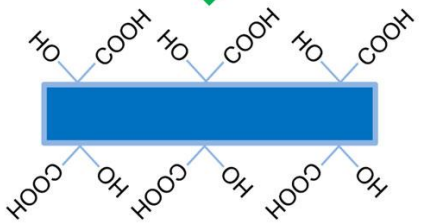


180 000 tons/y

PLA



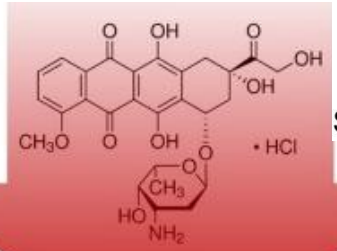
Cutinase from *Humicola insolens*

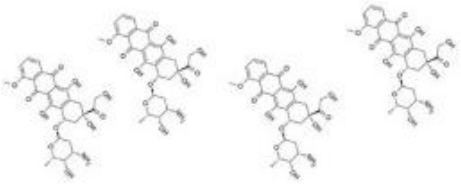


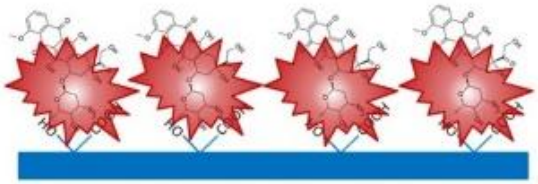
Surface-functionalized polyester

PLA

Doxorubicin







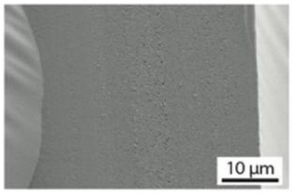
No adsorption

Adsorption

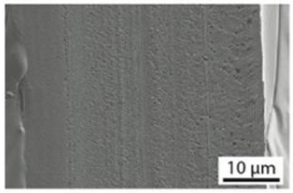
BOKU

Pellis A. et al., *Process Biochem.*, 59, 77-83, 2017.

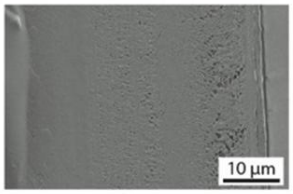
Cutinase hydrolysis of PLA preserves bulk properties



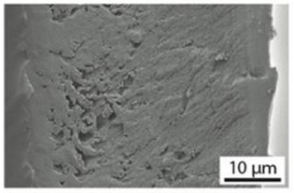
Start PLA



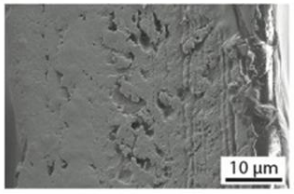
CTRL 48h



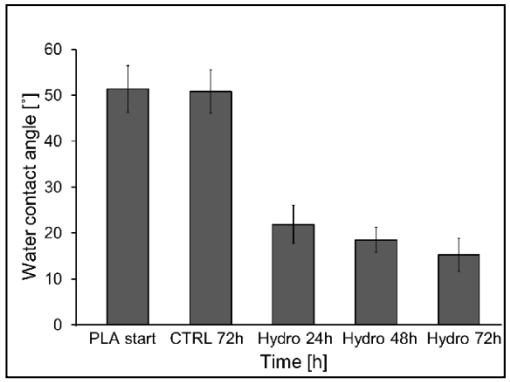
Hydro 24h



Hydro 48h



Hydro 72h



GPC

Treatment	M_n	M_w
Starting PLA	26493	188104
72h Enzymatic Hydrolysis	25874	181826
10min	18662	128248
20min	9466	51007
30min	6738	37827
60min	3984	18178

5 µM *Humicola insolens* cutinase

0.1 M NaOH

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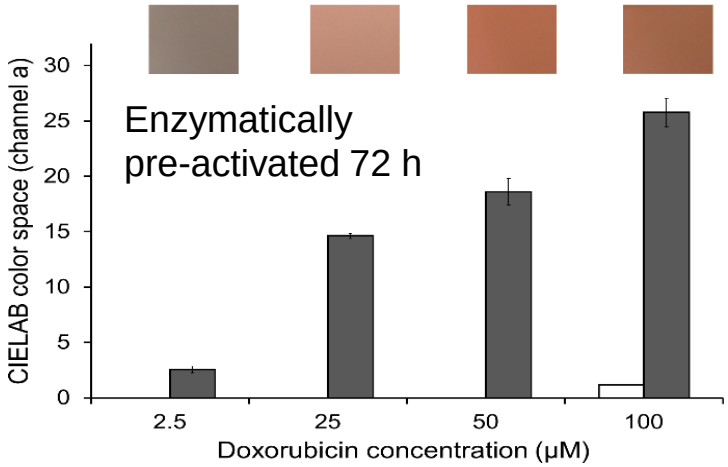
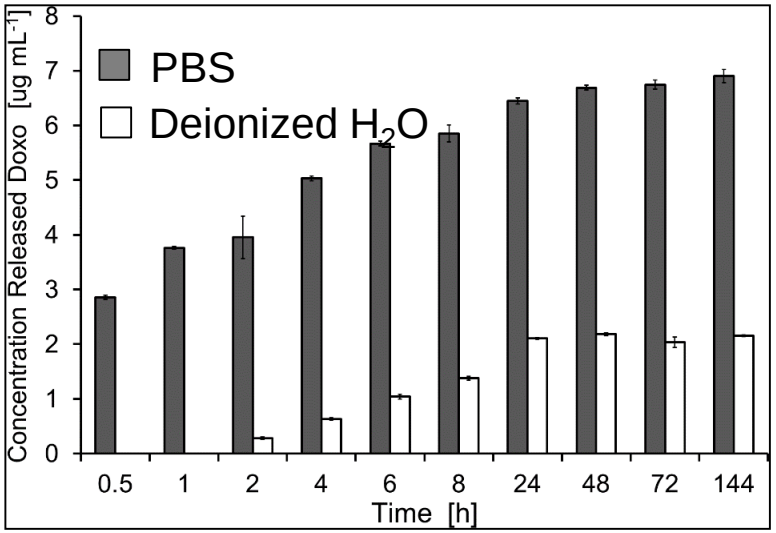
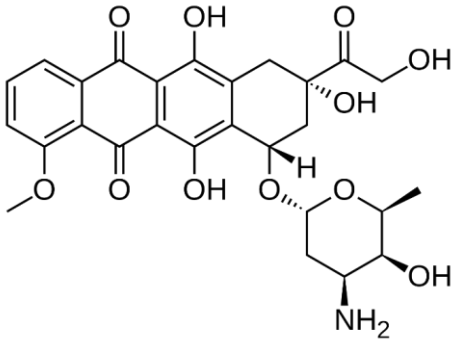
Photometric and FT-IR analysis of Doxorubicin adsorbed on PLA films

Adsorbed Doxo



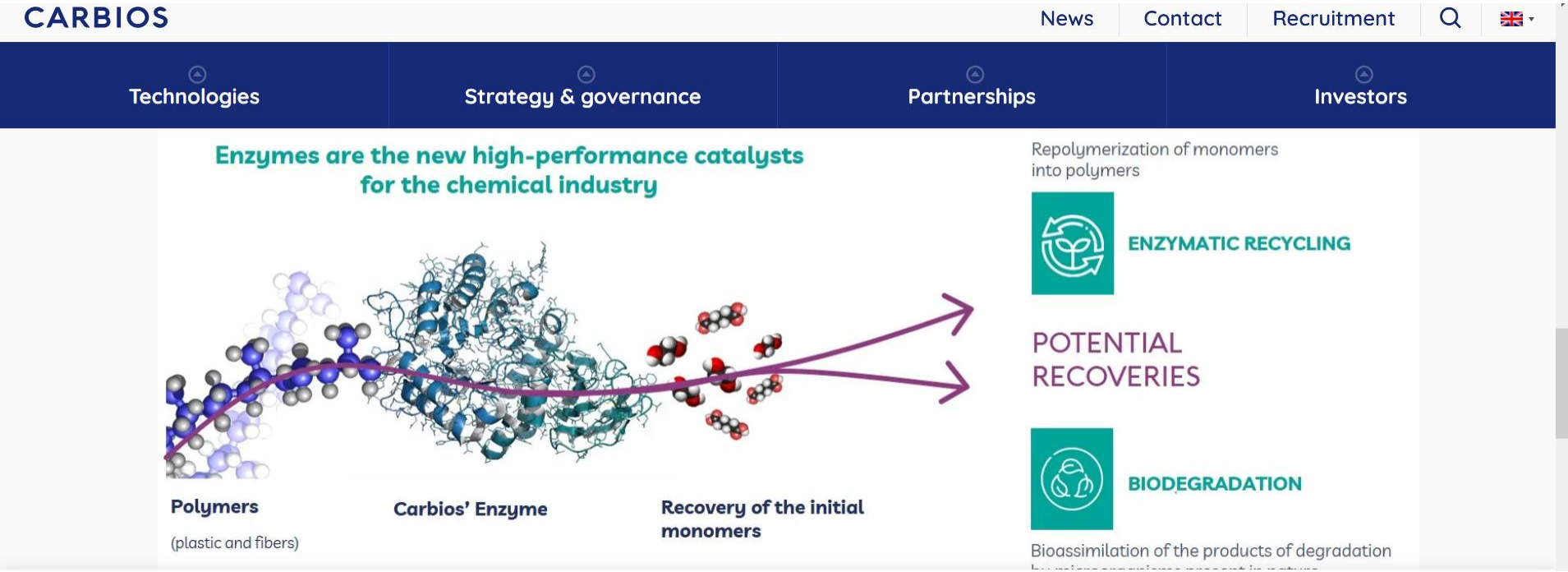
Released Doxo





Pellis A. et al., *Process Biochem*., 59, 77-83, 2017.

Biodegradazione, recupero e riciclo chimico dei monomeri (poliesteri)



Carbios, L'Oréal, Nestlé Waters, PepsiCo and Suntory Beverage & Food Europe