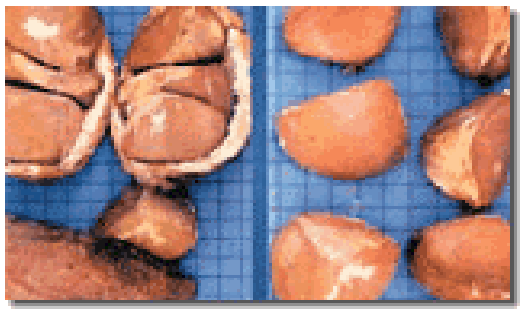
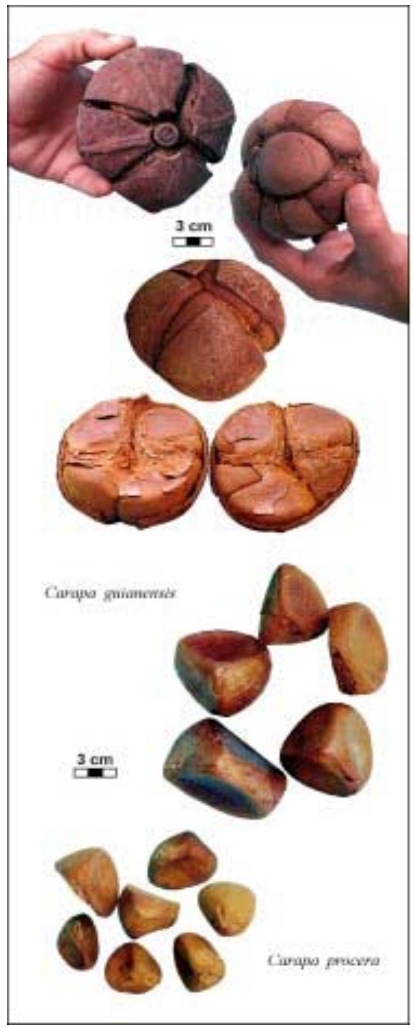
When considering the theme of our presentation, and thinking about the immense variety of raw-materials from where lipids could be extracted, as shown with the following few examples:

Soybeans (*Glycine maxima*)

Olives (*Olea europa*) and palm fruits (*Elaeis guineensis*)

Andiroba (*Carapa guianensis*)





Pupunha (*Bactris gasipaes*)

Brazil nuts (*Bertholletia excelsa*)

Babassu (*Orbignya martiana*)

Buriti (*Mauritia flexuosa*)

Açai (*Euterpes oleífera*)

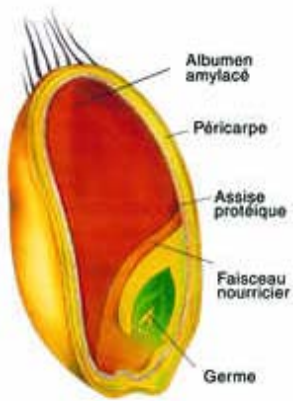
Castor beans (*Ricinus communis*)

Coffee seeds (*Coffea arabica*)

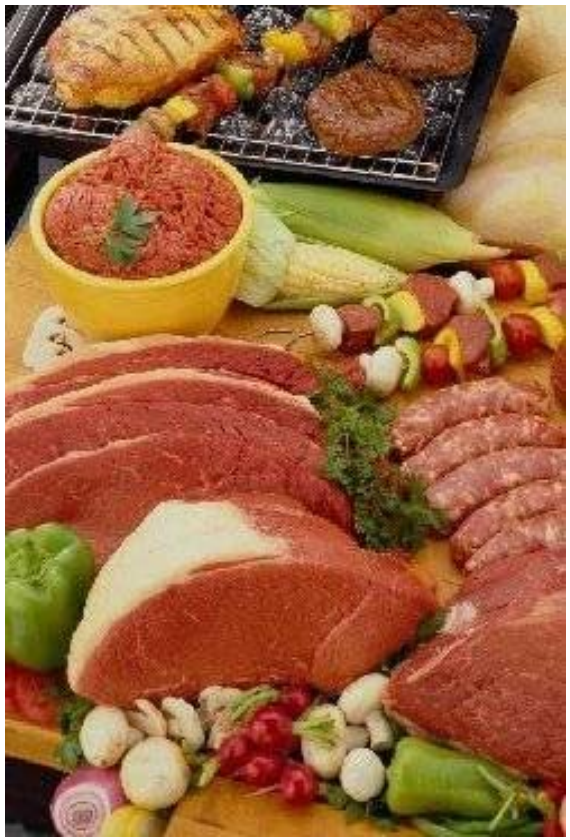
Cocoa (*Theobroma cacao*)

Avocado fruit (*Persea americana*)

Wheat (*Triticum sp.)*



Meat



Seafood

It comes inevitably a question:

- What is a lipid ?

- What is an oil/fat?

Lipids can roughly be considered

- Water insoluble compounds
- Soluble in non-polar solvents (Pet ether, Bz, $CHCl_3$)

comprising

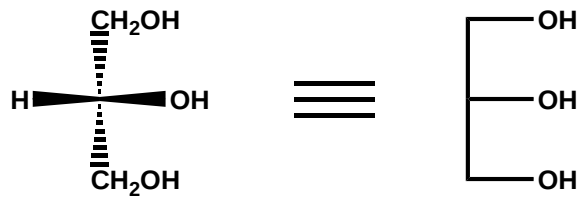
- Different types :
  - Fatty acids
  - Neutral lipids
  - Phospholipids and other lipids

Lipids can also be classified as:

Neutral
Polar
and Derived Lipids

1.Neutral lipids or Simple Lipids

- Acilglycerols (fats and oils); glycerides
  - Glycerol + fatty acids (>12 C)

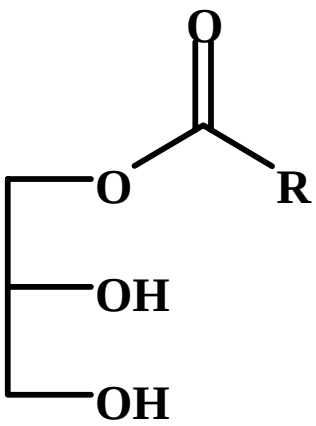


glycerol is a prochiral molecule

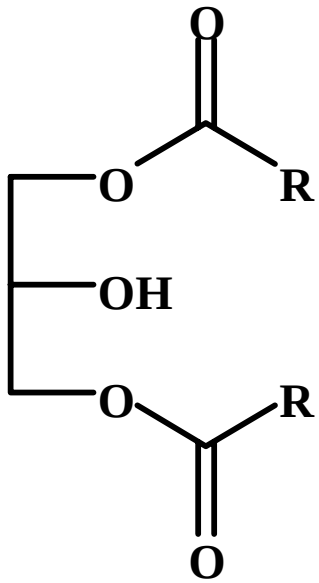
Esters of glycerol - mono-acilglicerols, di-acilglycerols and tri-acilglycerols

- Waxes – esters of long chain alcohols

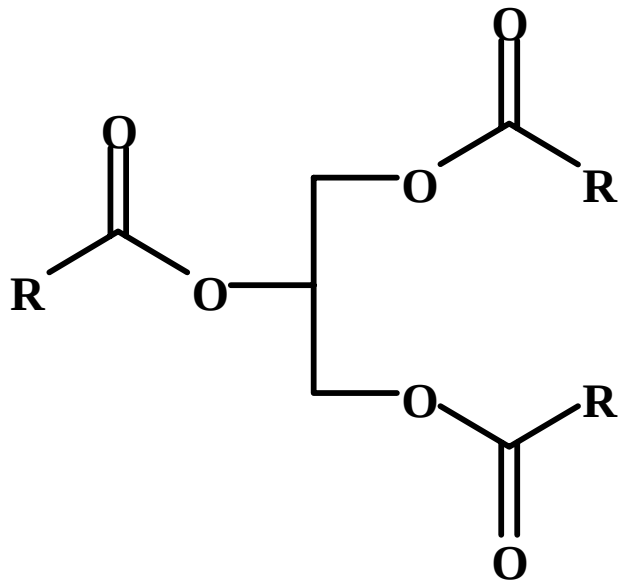
GLYCERIDES



MONOGLYCERIDE



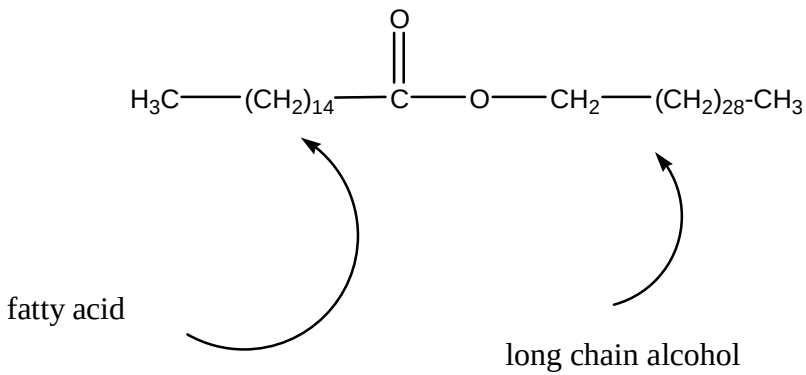
DIGLYCERIDE



TRIGLYCERIDE

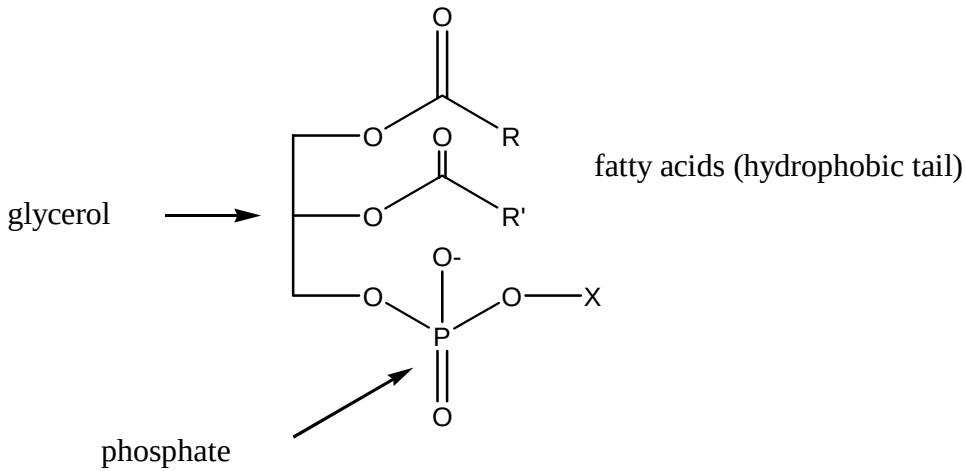
WAXES

- simple esters of fatty acids (usually saturated with long chain monohydric alcohols)



2.Polar Lipids

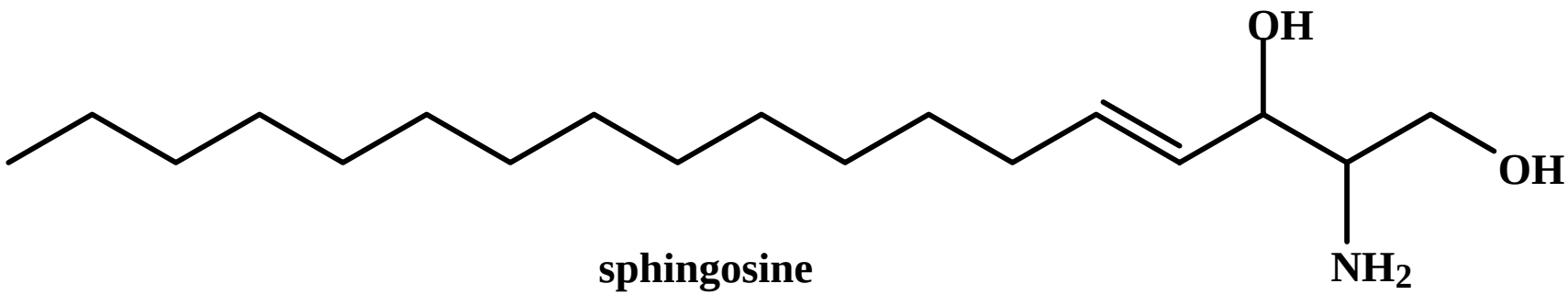
- **Phospholipids**: the major components of cell membranes
  - phosphoglycerides



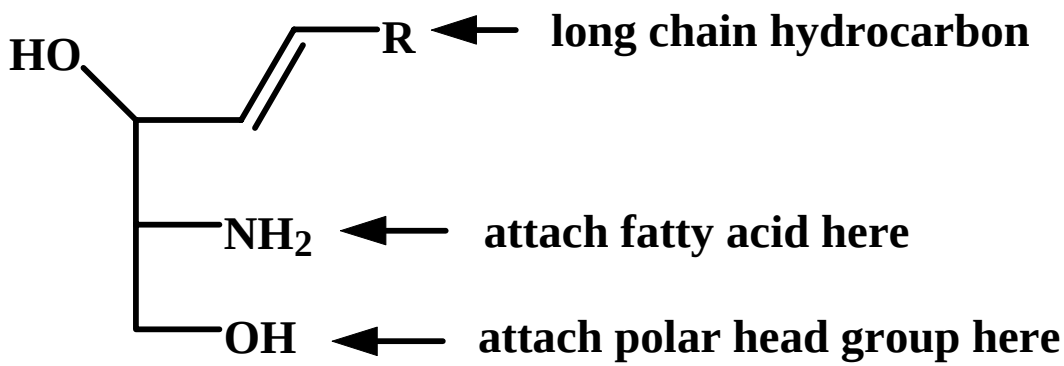
Phospholipids are generally composed of FAs, a nitrogenous base, phosphoric acid and either glycerol, inositol or sphingosine

2.Polar Lipids

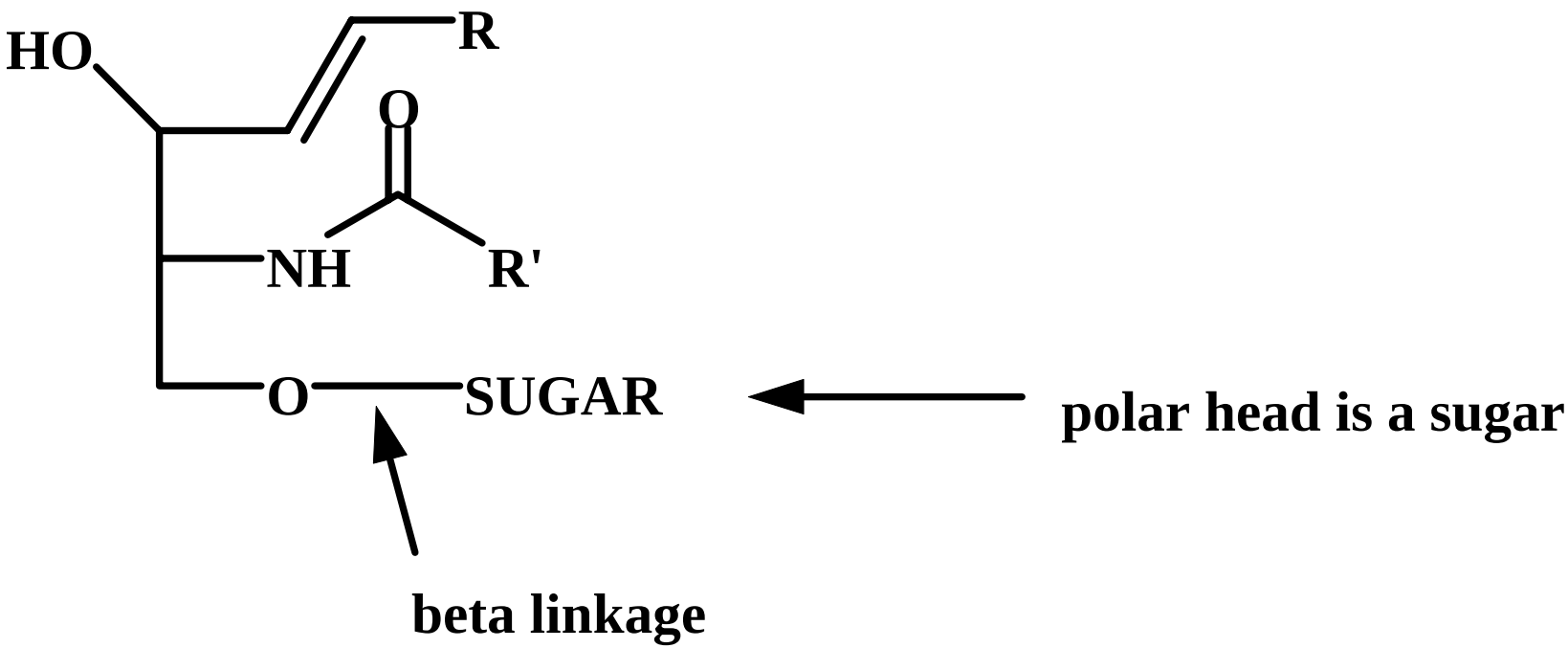
Sphingophospholipids: Based on sphingosine skeleton



sphingosine



2.Polar Lipids

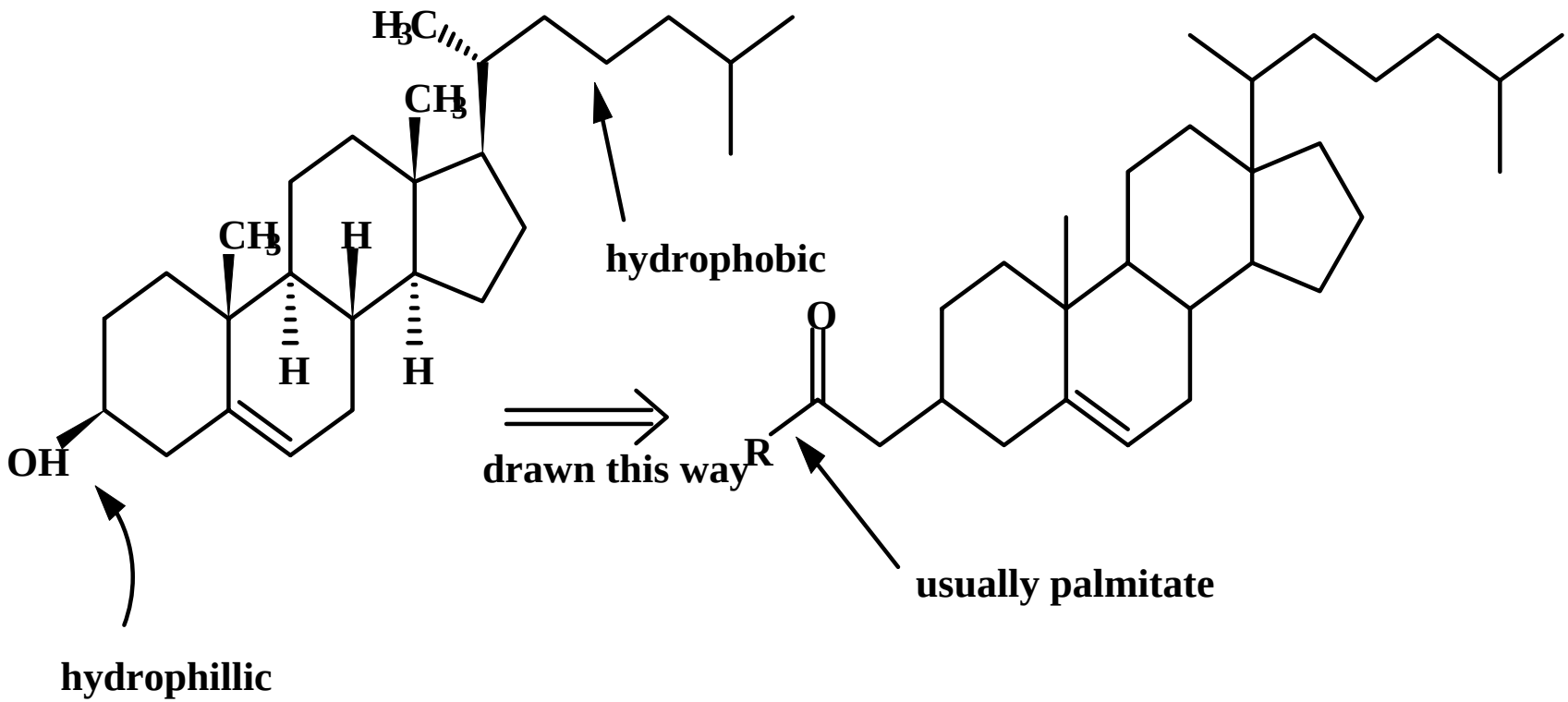


Glycolipids: Cerebrosides, gangliosides, lactosylceramides**…**

We also have Aminolipids, sulfolipids etc. groups of lipid compounds that in many cases are not well defined but contain amino acids, sulfur based compounds etc. Apart from tocopherols and quinone lipids – often considered as « redox lipids » - the so called Derived Lipids come from the previous groups by hydrolysis such as:

3.Derived lipids

(sterols and sterol esters)



In spite of the huge number of natural lipid raw materials to which we shall add all the lipid containing processed products and the possibility of crossing them with the various types of lipids which certainly exhibit different characteristics, what we finally consider as a **fat** appears rather simple:

Fat definition

.All substances extracted under the method conditions!

Other definitions can be found:

.*“total lipids including phospholipids”*

. *all the unchanged fatty acids from the food lipids converted to triacylglycerols (**net fat**)*

. *sum of all lipids expressed as triacylglycerols*

Now, we can go to the theme of our presentation

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LIPID EXTRACTION FROM DIFFERENT MATRICES

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Georges Piombo
Rosemar Antoniassi

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Embrapa Labex - France
Embrapa Food Technology, Rio de Janeiro, Brazil

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Embrapa

CIRAD

- Definition of fat
- Why to determine fat content
- The choice of the method
- Description of some methods
- Conclusions

Why to determine fat content?

- Raw-material composition
- Breeding programmes
- Process monitoring
- Medical research
- Nutritional labeling
- Business

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The choice of the method

- Methods using extraction with organic solvents – *Reflux methods*
- Combined methods - *Digestion step methods*
- Non-heating methods with purification step by washing (Folch type) or on dry column
- Physical methods

Obs. Some methods can be automated

The choice of the method

- The goal
- Quantitative or qualitative
- The raw material
- The urgency
- The price
- Availability of equipments and/or materials
- Repeatability
- Practicability
- Reproducibility – interlaboratorial analysis

- Regarding raw material:

- Non-bonded lipids

- Bonded lipids

The choice of the solvent

The solvent should be able:

- To extract all the lipid matter
- To be selective (to extract only the fatty material)
- To exclude degradation of the extracted material
- The working temperature should be able to reduce analyte (lipids) viscosity, increase the diffusion coefficients, and facilitate the solute desorption.
- Environmentally safe (preferably)

Solvent \ characteristics	b.p. °C	Dielectric constant 25°C	Toxicity
		Non-polar	
n-Hexane	69	1.89	
Cyclohexane	80.7	2.015	
Carbon tetrachloride	76.5	2.228	Low to Medium
Toluene	110	2.379	High
Ethyl ether	35	4.335	
Chloroform	61.7	4.806	Medium
Methylene chloride	39.7	9.08	
n-Butanol	117.2	17.1	
Iso-Propanol	82.4	18.3	May be toxic if consumed
1-Propanol	97.4	20.1	
Acetone	56.2	20.7	
Ethanol	78	24.30	
Methanol	65	32.63	High
Water	100	78.54	
		polar	

Lipids – non-lipids interaction

Interaction Compounds	Method
Hydrophobic interaction (E<2Kcal/mole) non-polar amino-acids (valine, leucine, isoleucine etc.) and lipid hydrophobic chain	Non-polar organic solvent such as Chloroform and Hexane
Ion-dipole interaction Non-polar amino-acids and phosphoric group of lipids	Non-polar organic solvent such as Chloroform and Hexane
Hydrogen Bonding (E=0.5-12Kcal/mole) Proteins functional groups or other non-lipid compounds and lipid amino-, hydroxyl-and carboxyl-groups	Polar organic compounds such as MeOH, EtOH or water (high dielectric constant)
Ionic bonding Non-extractable components (e.g. alkali ions) and phospholipids (e.g. triphosphoinositidyl) which might interact forming complex structures.	Impossible to extract in a neutral medium. Need for an acid or basic pH.

Non-chemical bonding between lipids and other compounds

Inclusion Compounds Formation

The classical example is between amilose helix and lisolecithin which can only be extracted with n-butanol : water solution

Low permeability of cellular wall

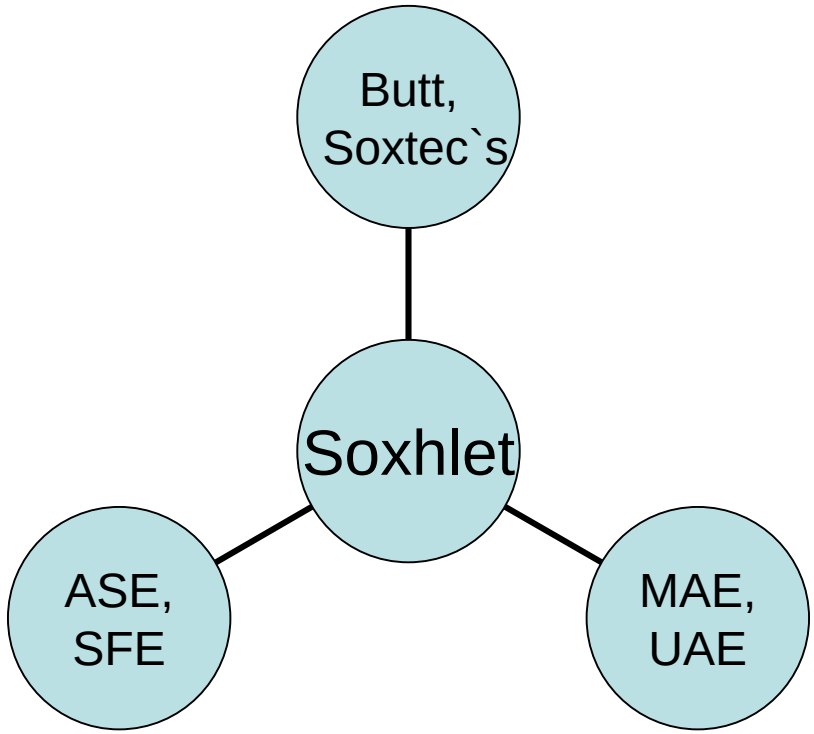
The solvent can not reach the lipid compound. Water, added to the extraction solvent, swells the polysaccharides making the cellular structure more permeable to the solvent.

The sample preparation

- Cleaning
- Pre-drying
- Grinding (“dry” or wet methods) and adjuncts

- The particle size
  - <2mm small seeds,
  - 4mm large seeds
  - 5-10µm (ASE)
  - 20-30mesh, depending on the seed

Solvent extraction methods



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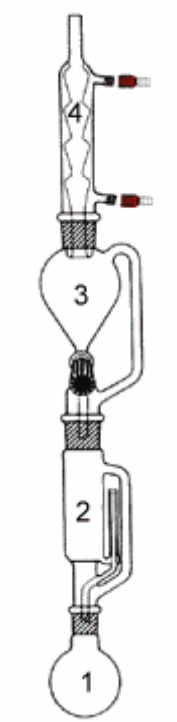


SOXHLET apparatus

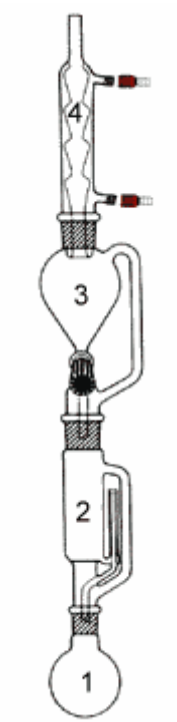
(1) flask containing the solvent
(and extract)

(2) extraction chamber with the thimble containing sample

(3) funnel to recover the solvent at the end of the extraction (unusual)

(4) condenser.

1 - Solid-liquid extraction *(Applicable to dried samples)*	
"Batch" extraction	*Solvent resides a certain time with the sample and siphoning is possible (e.g. Soxhlet extraction, 16h)*
Continuous extraction	*Percolation or through continuous washing of sample with the solvent (e.g. Butt extraction, 6h)*
Digestion	*Small amount of sample and a relatively large volume of solvent*
Liquid-Liquid Extraction *(Applicable to liquid and non dried samples)*	
Babcock (Gerbert) Method	*The fat is liberated with concentrated HCl or with acetic acid and H_2SO_4.*
Roese-Gottlieb (Mojonnier) Method	*Pre-digestion with NH_4OH, add ethanol and extract with pet.ether :ethyl ether 1:1.*



SOXHLET apparatus

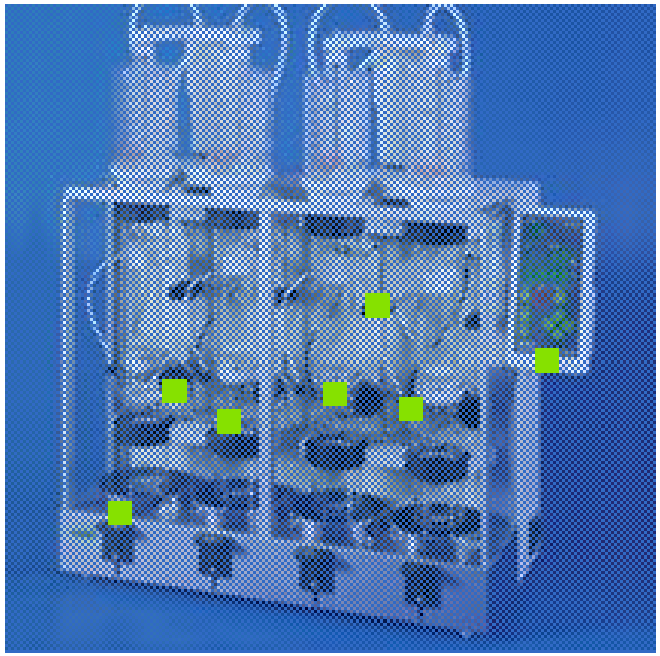
(1) flask containing the solvent
(and extract)

(2) extraction chamber with the thimble containing sample

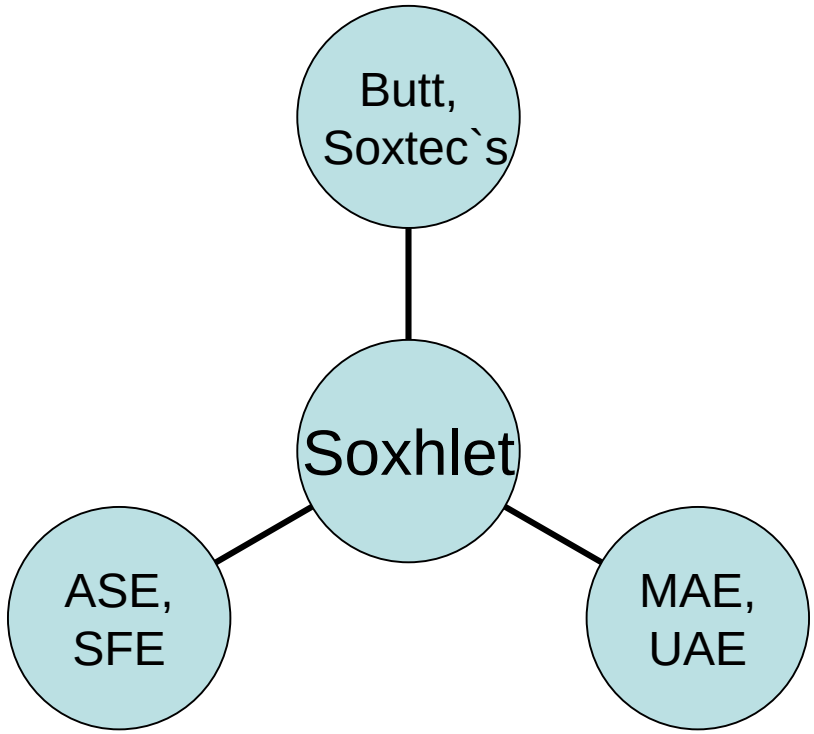
(3) funnel to recover the solvent at the end of the extraction (unusual)

(4) condenser.

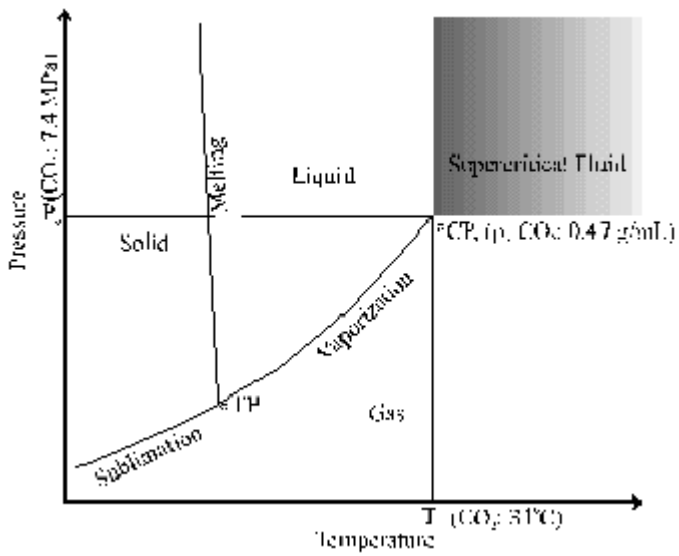
The Randall modification of Soxhlet apparatus, when the thimble containing sample is immersed into the solvent, was the base for different automated or semi-automated extraction instruments with heating blocks (as Büchi and Soxtec System) allowing fast determination of total lipids in different matrices, such as food and feed. These instruments perform boiling, rinsing and solvent recovery. We also find instruments able to perform acid hydrolysis coupled with an extraction system.



Solvent extraction methods



The supercritical fluid extraction resembles Soxhlet extraction except that the solvent used is a supercritical fluid (mainly SC CO_2), substance above its critical temperature and pressure.



SCF phase diagram, the supercritical region above the critical pressure (Pc) and temperature (Tc). CP = critical point, TP = triple point.

Advantages of SC CO_2

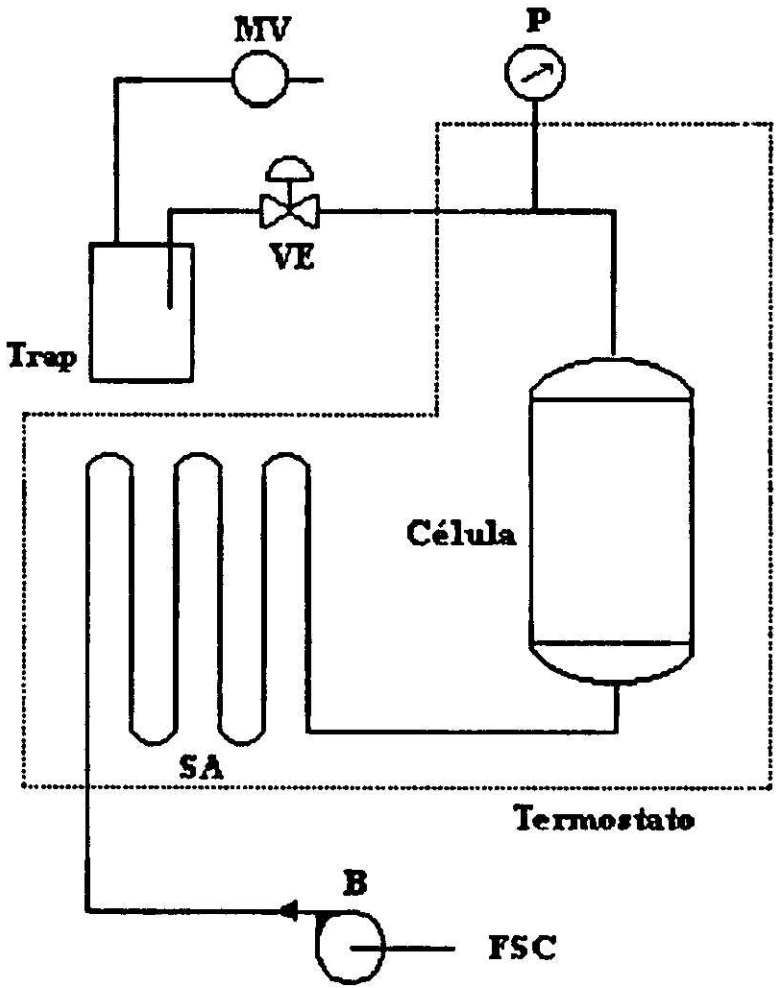
- **Lower viscosity**
- **Higher diffusion coefficient of lipids**
- **Higher compressibility coefficient**
- **Highly selective**
- **Faster extraction rates**
- **Less degradation of solute, particularly oxidation**
- **Non-inflammable**
- **Ecological benefits since eliminates organic solvents, its storage and disposal**
- **The cost is low (by-product from whisky distilleries, e.g.)**

Disadvantages of SCFE

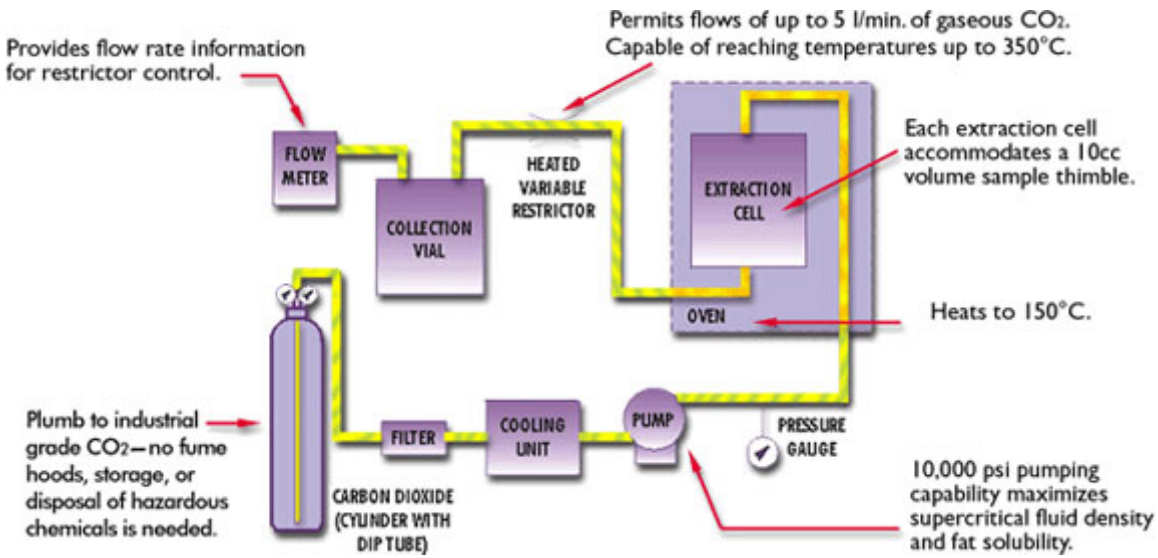
- Many parameters to optimizing:
  - Pressure
  - Temperature
  - Addition of organic solvent
- Specific conditions for each matrix
- Energy consumption

Typical FSCE Process

Dynamic Extraction Method



TFE2000 Flow Diagram Leco Co.)



In the Accelerated Solvent Extraction-ASE-, or Pressure Solvent Extraction, pressures (100 to 200bars) are applied permitting to maintain the solvent at higher temperatures making easier the penetration of the solvent thoroughly the matrix and thus accelerating the extraction.

Accelerated Solvent Extractor

Load sample into cell

Fill cell with solvent

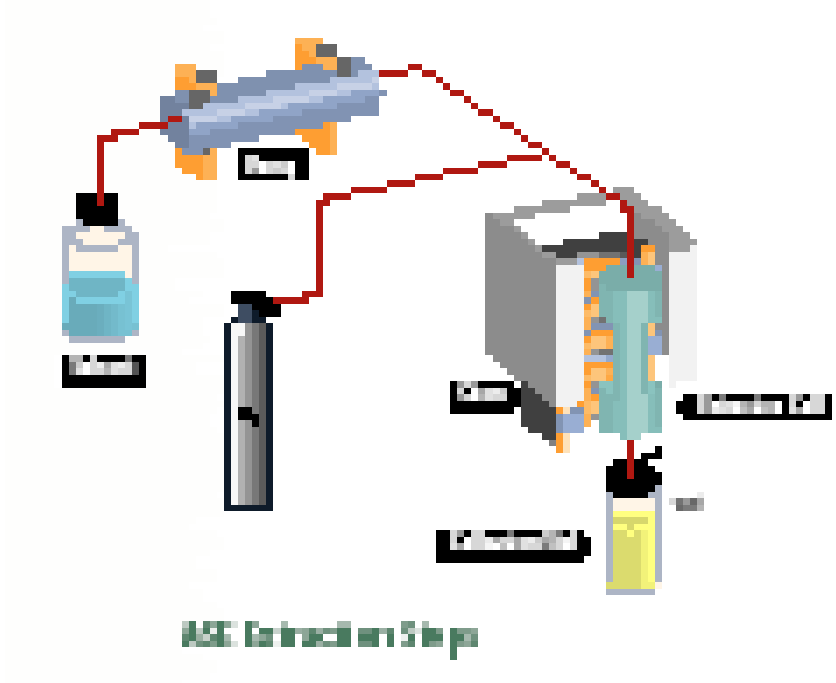
Heat and pressurize cell

Hold sample at pressure and temperature

Pump clean solvent into sample cell

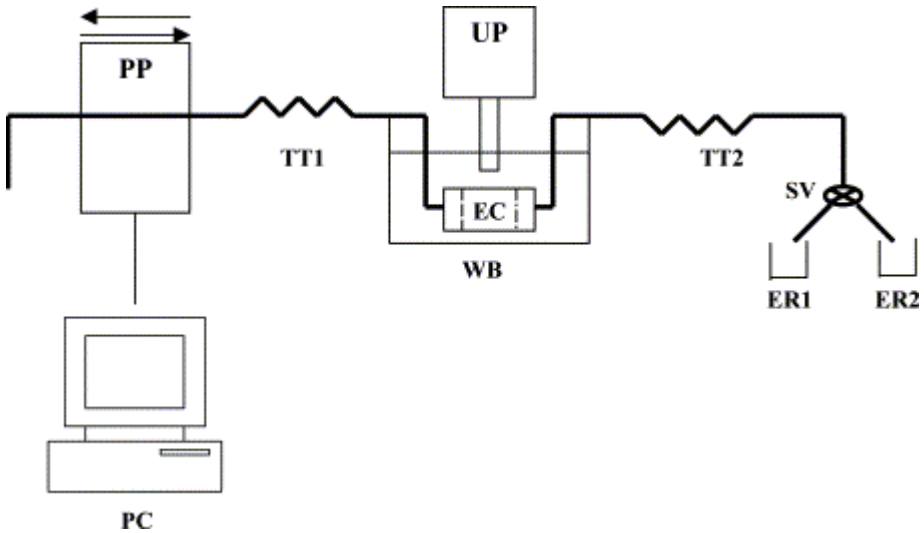
Purge solvent from cell with N_2 gas

Extract ready for analysis



Accelerated Solvent Extractor

The so called physical methods for fat measurements (principally NIR and NMR) were not included in our presentation but we should mention two recent advices for lipid extraction: MAE – Microwave assisted extraction, where the heating is provided by microwave irradiation and UAE-ultrasound assisted extraction method aimed mainly at bakery products total fat content determination



Dynamic UAE of total fat content. PP, peristaltic pump; UP, ultrasonic probe; EC, extraction chamber; WB, water bath; ER1 and ER2 extract and extractant reservoirs; TT, transport tube; PC, personal computer; selection valve. (Ruiz-Jiménez and Luque de Castro. 2004)

Summary

Method	Principle	Scope of application	Advantages	Limits
Reflux	-Grinding and drying -Reflux extraction (hexane, pet. or ethyl ether, EtOH)	Solid, semi solid or dry products (seeds, meat, feed, powder milk)	Quantitative Repeatable Standardized Automated	Long protocols Non extraction of polar lipids Not for humid or liquid products No purification steps
Acid hydrolysis + gravimetry	Hot hydrolysis with HCl, followed by extraction	Dairy, Meat and vegetable products Feed Cereal	Standardized methods Polar lipids extraction	Long protocols Sugar drawning TAGs hydrolysis Strong acids manipulation
Alkaline hydrolysis + gravimetry	Hydrolysis with ethanolic NH4OH -Extraction with ethyl ether and pet ether (Rose-Gottlieb method)	Milk and dairy products	Standardized methods Polar lipids extraction	TAGs hydrolysis Loss of fa in aqueous phase
Cold + rinsing purification	Folch CHCl3:MeOH 2:1 Bligh& Dyer (CHCl3 : MeOH : H2O 1 :2 : 0.8) and others	Biological extracts Meat, fish Any food product	Reference biochemical method (Folch) Polar lipids extraction No degradation Suitable for any matrix	Sugars and hydrophobic proteins drawning Emulsions can appear during washings Toxic solvent Loss of hydrossoluble lipids
Cold + dry column purification	Maxwell method Dispersion with Na2SO4 + Celite 545 +CaHPO4 Extraction by elution through a column with CHCl2:MeOH 9:1 (also with hexane:IspOH 3:2)	Any food product	Standardized methods Polar lipids extraction No degradation	Possible drawn of sugars Slow elution if water Celite has to be neutral
	Based on Castera-Rossignol, 1998.			

Comparative data

Device	Price (euros)	Time	Solvent Volume	Price/sample (for 2000analyses/y.)
Soxhlet	600	16h 4-48	200-500ml	1.9
Butt	500	6-8	<500ml	<1.9
Soxhlet automated		1-4	50-100ml	1.1
SFE	20,000 to 30,000	30min-2h	8-50ml	1.6
ASE	45,000	12-18min	15-40ml	1.0
Ultrasound Assisted Extraction		30min-1h (snacks) 3h (cookies)	100-300ml	1.7

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Conclusions: what method to use?

- Validated methods (AOCS, ISO, IUPAC, DGF, AFNOR, IOOC, FOSFA etc.)
- According to your goal and facilities
- According to your raw material
- Respecting the environment

since

- **There is no an ideal method for fat determination**

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CIRAD

THANK YOU FOR YOUR

ATTENTION!

regina.lago@cirad.fr

References

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- Luthria, DL. Oil Extraction and Analysis. 2004. AOCS press. 282p.
- Rossignol-Castera, A. 1998. Un préliminaire important: l'extraction analytique des lipides. OCL, v.5, 4:300-305.
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