THIN-LAYER CHROMATOGRAPHY OF LIPID FRACTION IN TREE NUTS SPECIES

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Nuts and their nutritional importance

- Nuts are defined as fruits with less than 50% of water.
- This definition involves Almonds, Cashews, Hazelnuts, Walnuts, Peanuts, Pistachios, Brazilnuts, Pine nuts and Pecans.
- Nut tree species are situated in very distant sites in vegetal Kingdom.

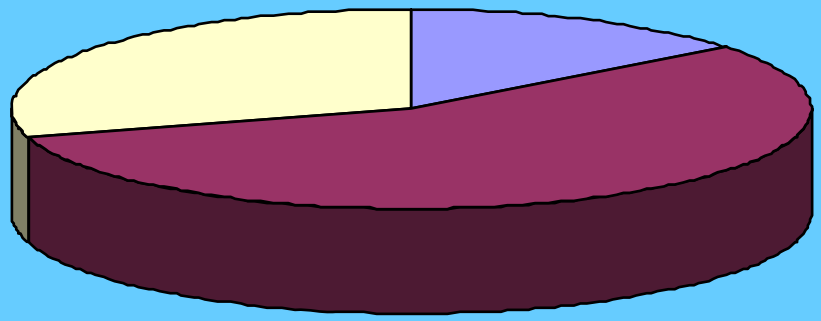
	Almonds	**Cashews**	**Hazelnuts**	**Walnuts**	**Peanuts**	**Pistachios**	**Pine Nuts**	**Pecans**
Order	Rosals	Sapinals	Fagals	Juglandals	Fagals	Sapinals	Coniferals	Juglandals
Gender	Prunus	Anacardium	Corylus	Juglans	Arachis	Pistacia	Pinus	Carya
Specie	Dulcis	Occidentale	Avelana	Regia	Hypogaea	Vera	Pinea	Illinoinensis

Nuts and their nutritional importance

• Nuts have a high portion of fat (45-75%), however Nuts are considered one important piece in healthy diets.

• Fat from Nuts have a degree of saturation near to ideal intake in humans.

• Their high portion of MUFA (mono unsaturated fatty acids) makes Nuts an excellent food to weight control and decrease cardiovascular risk.



SATURATES MONOINSATURATES POLIINSATURATES

Nuts and their nutritional importance

Nuts have also considerable amounts of many compounds involved in human wellness:

- Antioxidant Molecules
- Phitosterols

In processing plants Nuts go through severe transformation processes.

(heating, frying, etc…)

Lipid analysis in nuts

Lipids are a big cluster of compounds with a wide range of structures.

Lipids can be classified in two groups according to the presence/absence of fatty acids.

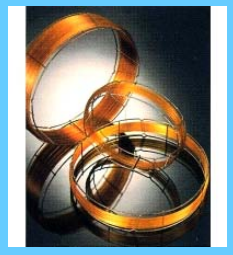
SAPONIFICABLES (With fatty acids)	**UNSAPONIFICABLES** (Without fatty acids)
PHOSPHOLIPIDS	**PHITOSTEROLS**
GLYCERIDES	

Lipids have two main roles in cells: source of energy and structural function in cell membranes.

Lipid analysis in nuts

The Key technique to analyze lipids is Chromatography

GC



CHROMATOGRAPHY

HPLC

TLC/ HPTLC

Principal advantages of TLC and HPTLC

• **Economy:** Methods of thin layer chromatography are much cheaper than HPLC methods.

• **Versatility:** Same plates and same systems of revelation and quantification are valid for analysis of a wide range of lipid types.

• **Speed:** The property of TLC systems of carrying a large number of samples in one plate makes the time necessary for each sample minimum.

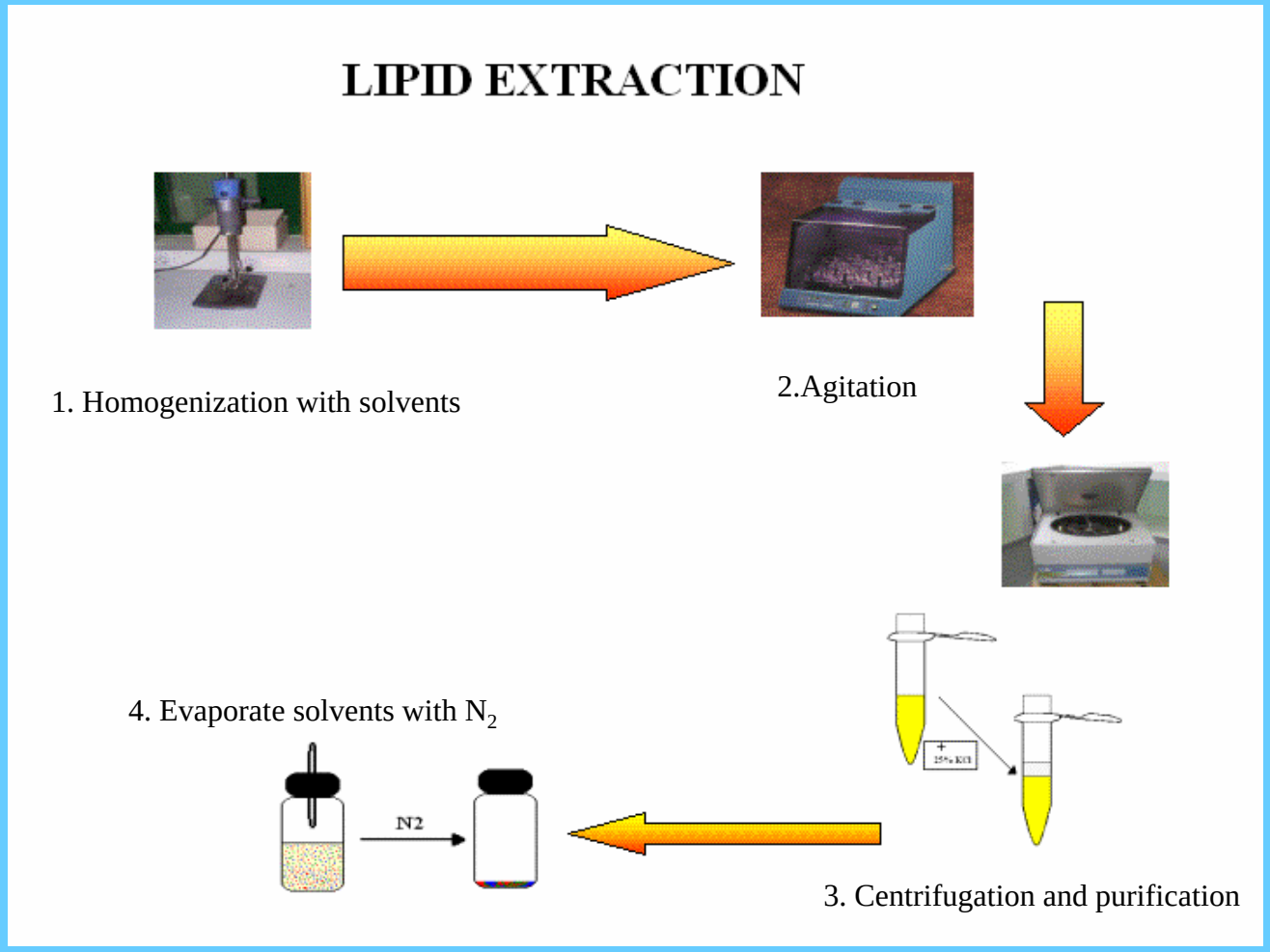
Samples Selection

Selected samples are: Almonds, Hazelnuts, Chestnuts, Walnuts, Peanuts, Commercial homogenate of peanuts and Pistachios.

Samples are purchased in BORGES SA and maintained under standard conditions before being analyzed.

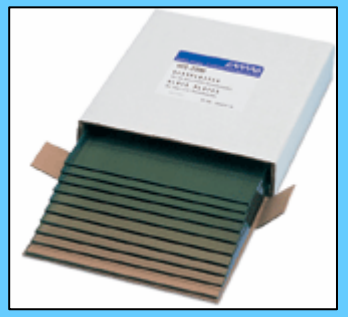
Extraction of lipids in Nuts

Extraction about Folch method with modifications



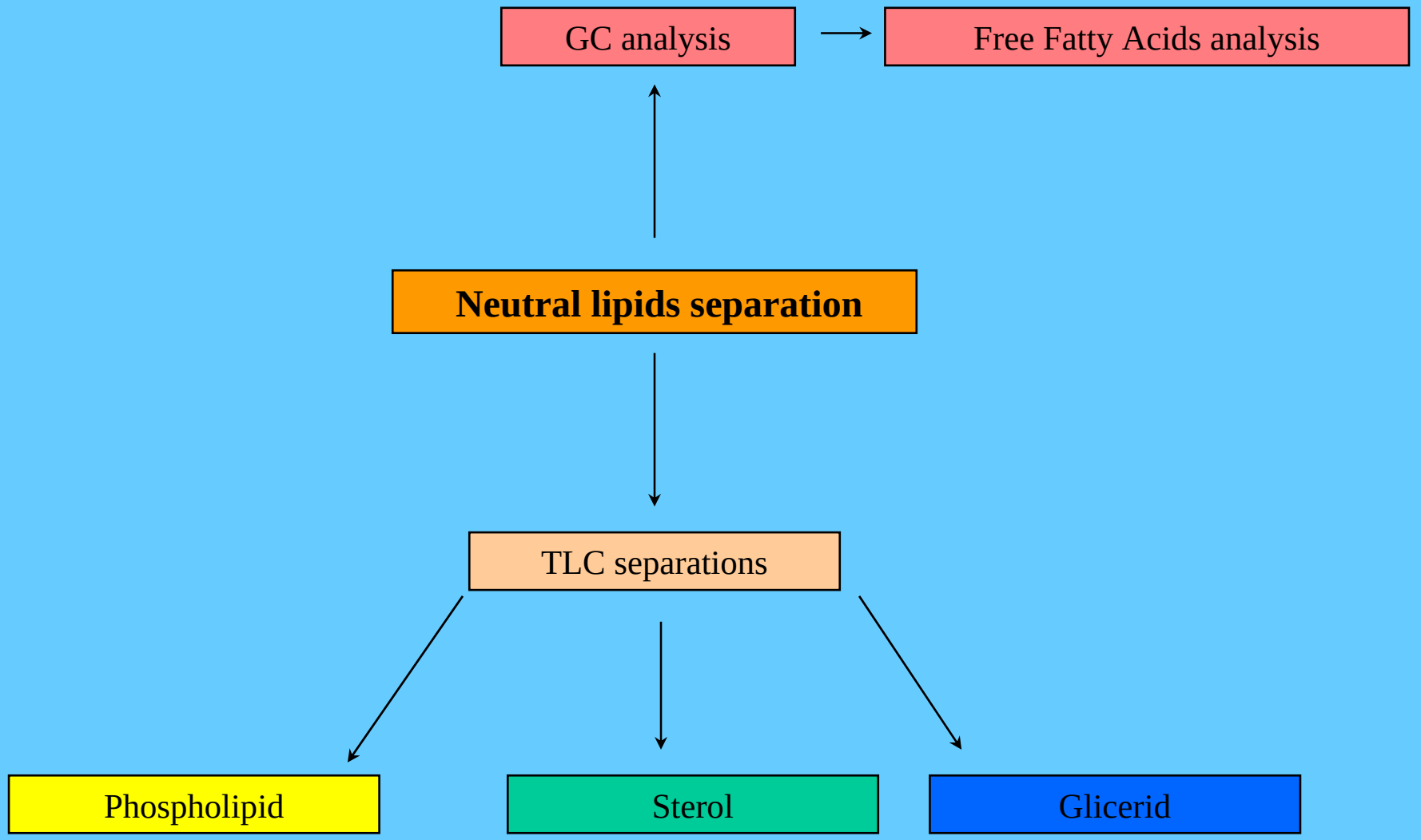
Plate’s and Sampling Selection

Plates used are MERCK made of
Silica Gel 60F256



The sampling method selected was
micro capillaries with holder of 2 ìl

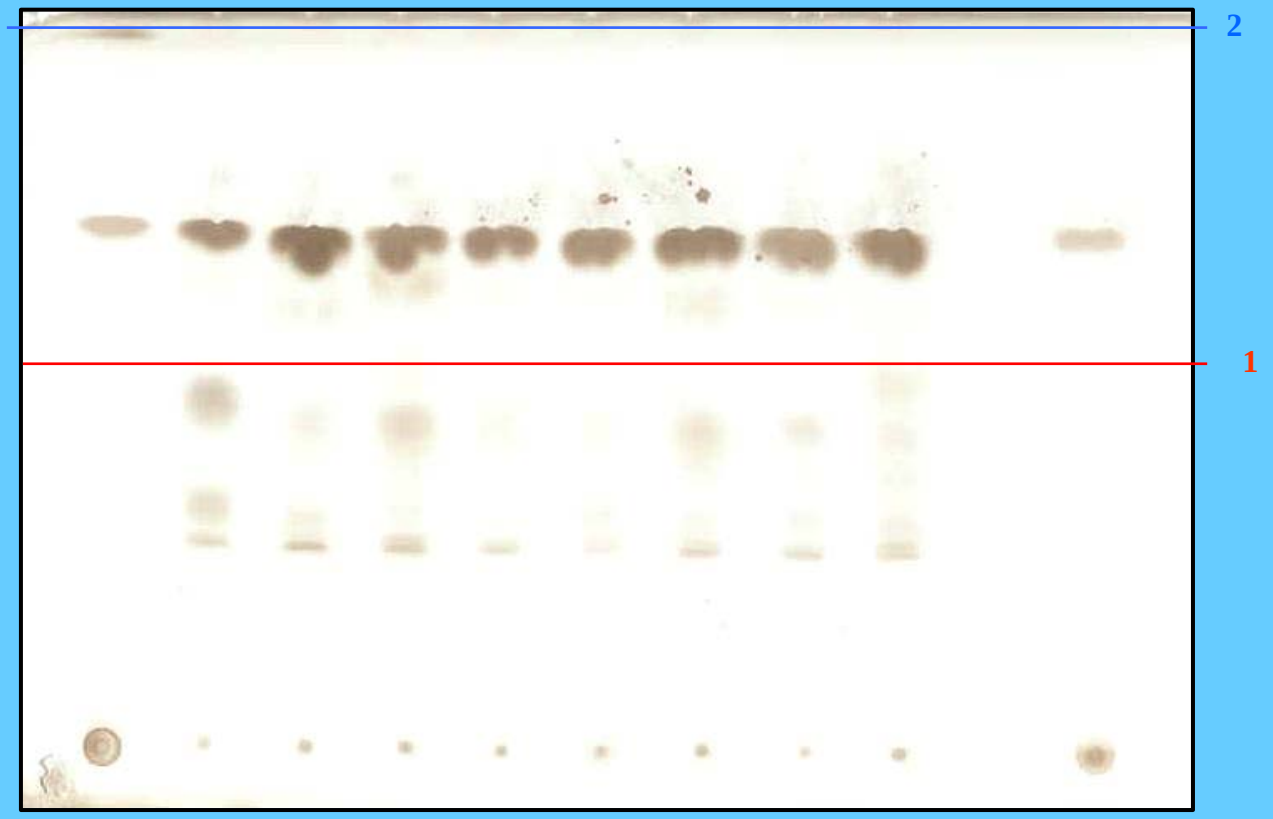
Steps of complete lipid analysis in nuts



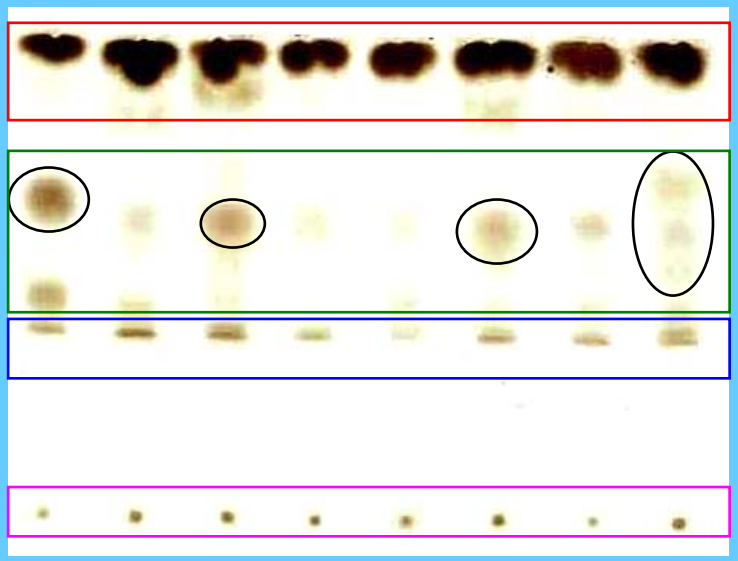
First separation of Neutrals Lipids

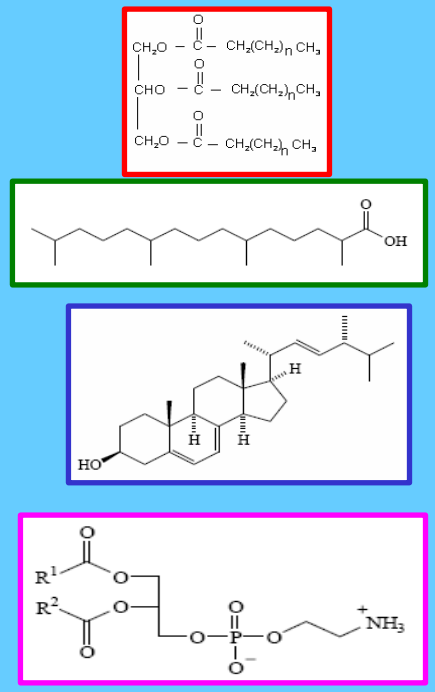
SOLVENT SYSTEM: Two solvent system development: Hexane, TBME, Acetic Acid (70:30:2) [1] Hexane [2]

REVELATION SYSTEM: Spraying 10% $CuSO_4$ in 8%H_3PO_4 heating in plate at 180º 30‘



Neutral lipids of Nuts





GLICERIDS

FREE FATTY ACID

STEROLS

PHOSPHOLIPIDS

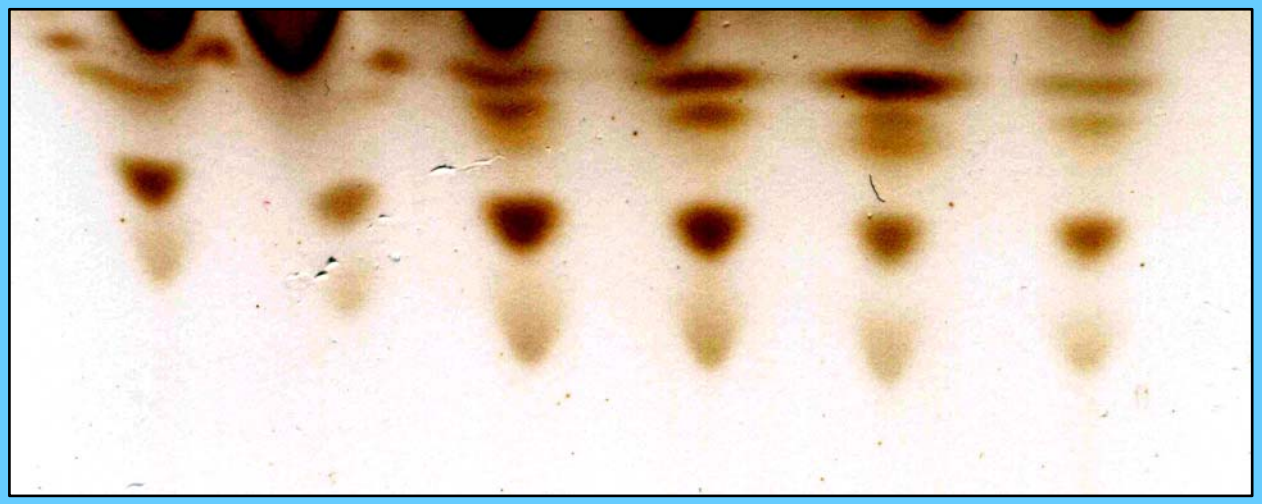
- All samples have a similar pattern of Neutral lipids.
- Glycerides are the most abundant form of lipids in nuts.
- Some nuts (Comercial homogenate of Peanut, Almond and Hazelnut) have a higher proportion of free fatty acids.

Ch: Chestnuts **Hz**: Hazelnuts **Hc**: Commercial homogenate of Peanut **Al**: Almond **Wl**: Walnut **Pn**: Peanut **Ps**: Pistachio

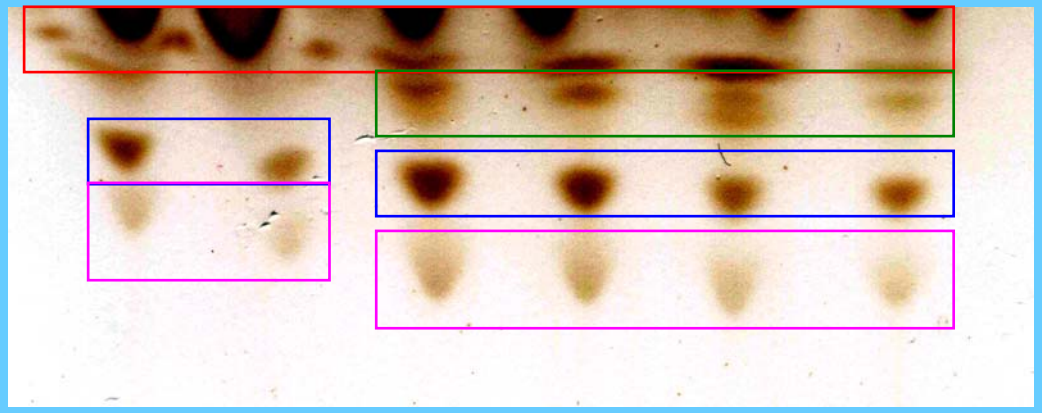
Phospolipid separation

SOLVENT SYSTEM: Hexane, TBME, Acetic Acid (70:30:2)

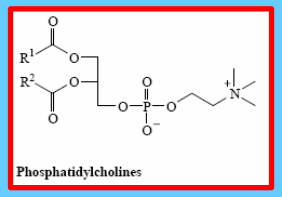
REVELATION SYSTEM: Spraying 10% $CuSO_4$ in 8%H_3PO_4 heating in plate at 180º 30‘



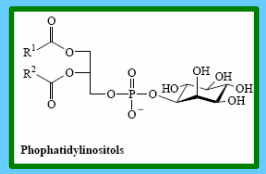
Phospolipids in Nuts



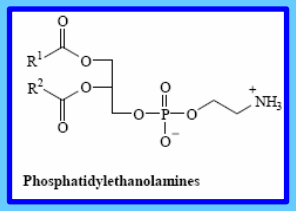
Hz Ps Pn Ch Wl Al



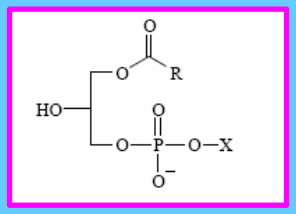
PHOSPHATIDYLCHOLINES



PHOSPHATIDYLINOSITOLS



PHOSPHATIDYLETHANOLAMINES



LYSOPHOSPHOLIPIDS

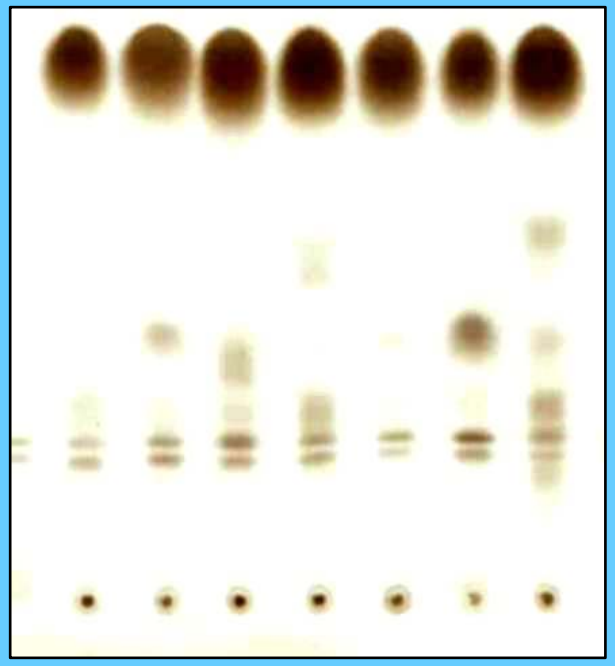
- Phosphatidylethanolamines are the most abundant Phospholipid in nuts.
- Hazelnuts and Pistachios have less Phosphatidylcholines than the other analyzed nuts.

Ch: Chestnuts **Hz**: Hazelnuts **Hc**: Commercial homogenate of Peanut **Al**: Almond **Wl**: Walnut **Pn**: Peanut **Ps**: Pistachio

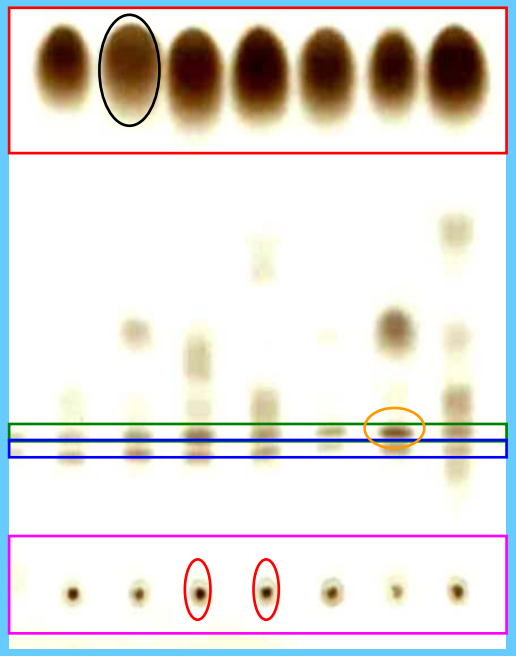
Glicerid separation

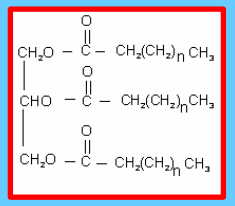
SOLVENT SYSTEM: Hexane, TBME, Acetic Acid (70:30:2) [1]

REVELATION SYSTEM: Spraying 10% $CuSO_4$ in 8%H_3PO_4 heating in plate at 180º 30‘

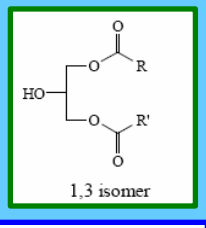


Glicerid separation of Nuts

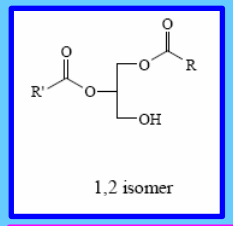




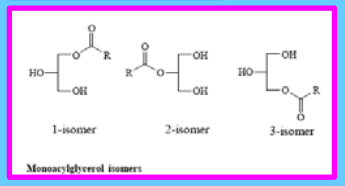
TRIACYLGLICEROLS



1-3 DIACYLGLICEROLS



1-2 DIACYLGLICEROLS



MONOACYLGLICEROLS

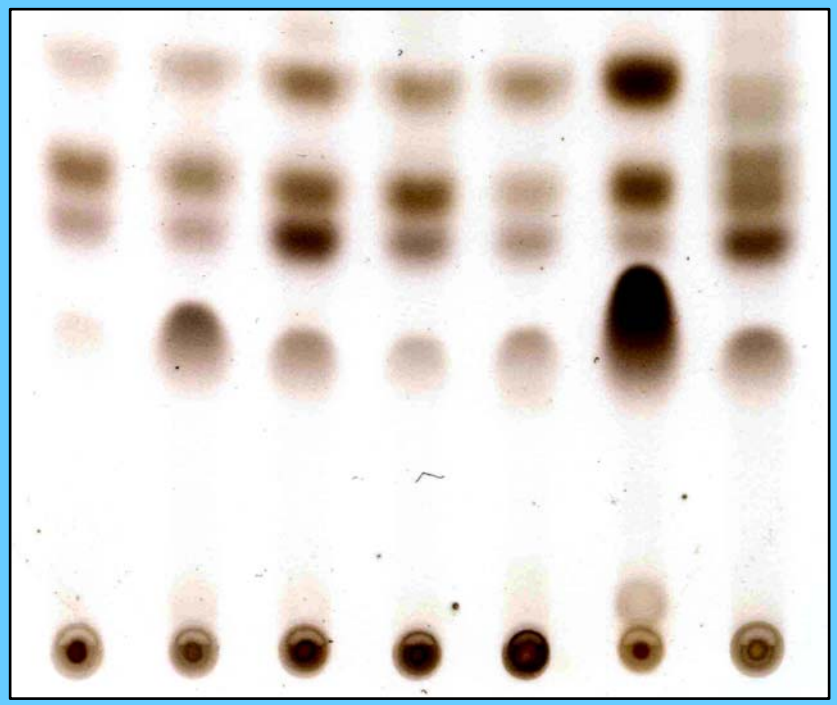
- The major components of glicerids in nuts are Triacylglicerols.
- Walnut has more 1-3 dyacilglicerols than other nuts.
- Peanuts and Pistachios are richer in Monoacylglicerols.

Ch: Chestnuts **Hz**: Hazelnuts **Hc**: Commercial homogenate of Peanut **Al**: Almond **Wl**: Walnut **Pn**: Peanut **Ps**: Pistachio

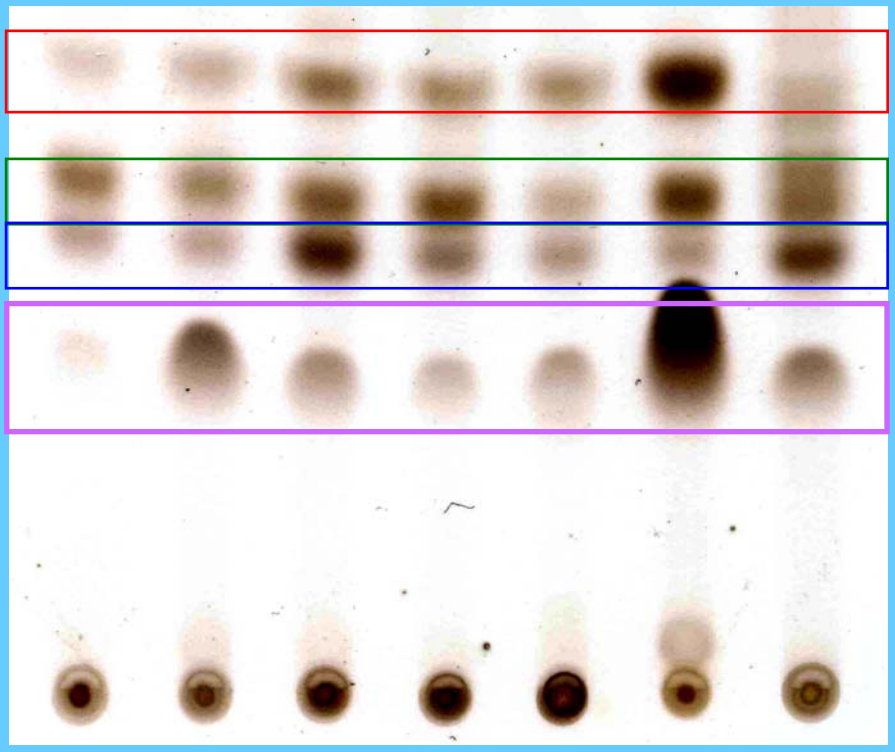
Sterol Separation

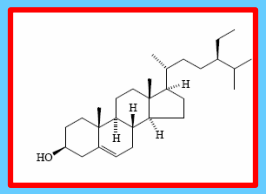
SOLVENT SYSTEM: Hexane, TBME, Acetic Acid (70:30:2)

REVELATION SYSTEM: Spraying 10% $CuSO_4$ in 8%H_3PO_4 heating in plate at 180º 30‘

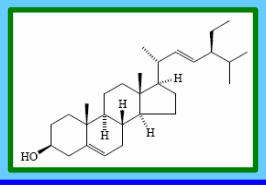


Sterols in Nuts

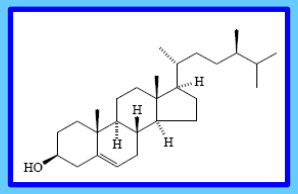




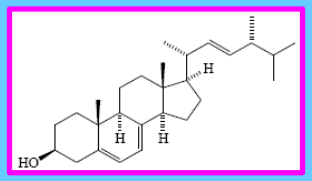
SITOSTEROL



STIGMASTEROL



CAMPESTEROL



ERGOSTEROL

- Walnuts have a higher portion of Phitoesterols.
- Pistachios and Almonds are richer in Campesterol.

Ch: Chestnuts **Hz**: Hazelnuts **Hc**: Commercial homogenate of Peanut **Al**: Almond **Wl**: Walnut **Pn**: Peanut **Ps**: Pistachio

What can HPTLC add to this study?

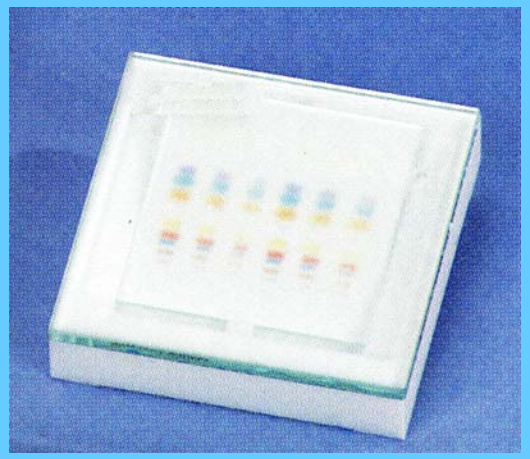
More resolution

↓

Precise quantification

↓

More accuracy in effects detection



- More samples in a plate.
- Newest and more complex separations.

Conclusions

• TLC systems are valid methods to analyze lipidic patterns in nuts

• TLC methods provide enough resolution to if the previous extraction is clean of non-lipidic components.

• HPTLC can be a good option to improve this type of analysis.

The Group of Research

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