LIPID ANALYSIS(Ch.13-14)

I. Quantification of lipid components

a.Extractions (wet vs dry ;solid-liq vs liq-liq.)

b.Indirect methods (NMR, X rays, Conductance)

II. Assays for further investigations on extracted oil

a.Purity criteria: give indications of wholesomeness or adulteration (physical:n_D^{20},density; chemical: IV, SV, FAME, sterols, tocopherols compositions)

b.Quality criteria: indicates good quality or deteriorated quality

b.1.MIU (Moisture, Impurities, Unsaponifiables)

b.2.Rancidity : Hydrtolytic:AV and ffa

Oxidative:PV,TBA,TOTOX etc

b.3. Storage stability (shelf-life)

Lipids: Fats and fat like compounds(not polymers) that are soluble in non-polar organic solvents(petroleum ether), but insoluble in H_2O. Lipids have:

1. Culinary importance: Great effects in preparation of foods simnce they provide heat transfer media, carry flavours, and contribute to palatability(eating quality) of foods.

2. Nutritional importance: Provide bulk of calories in diet (1 g: 9 kcal). For healthy diets, %30 percent of total calories have to come from lipids.

3. Physiological importance: They are vital for biological functioning of cells, since they contain essential fatty acids and oil-soluble vitamins.

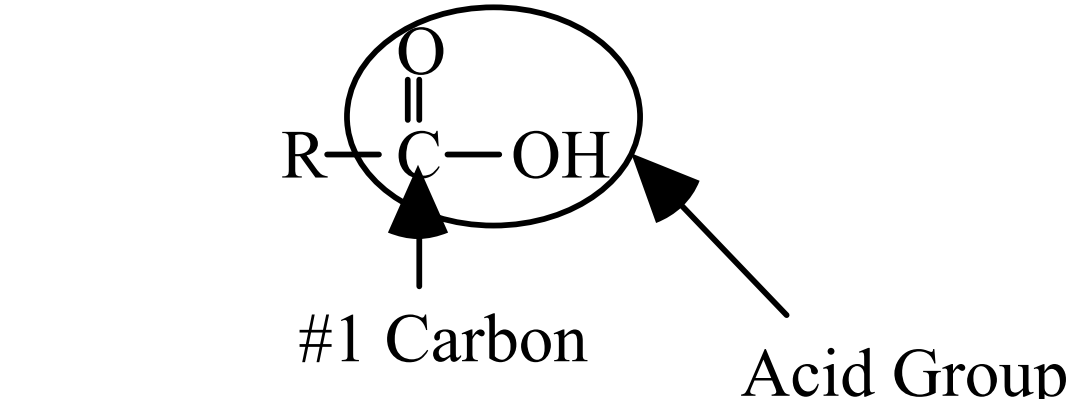
“LIPID”

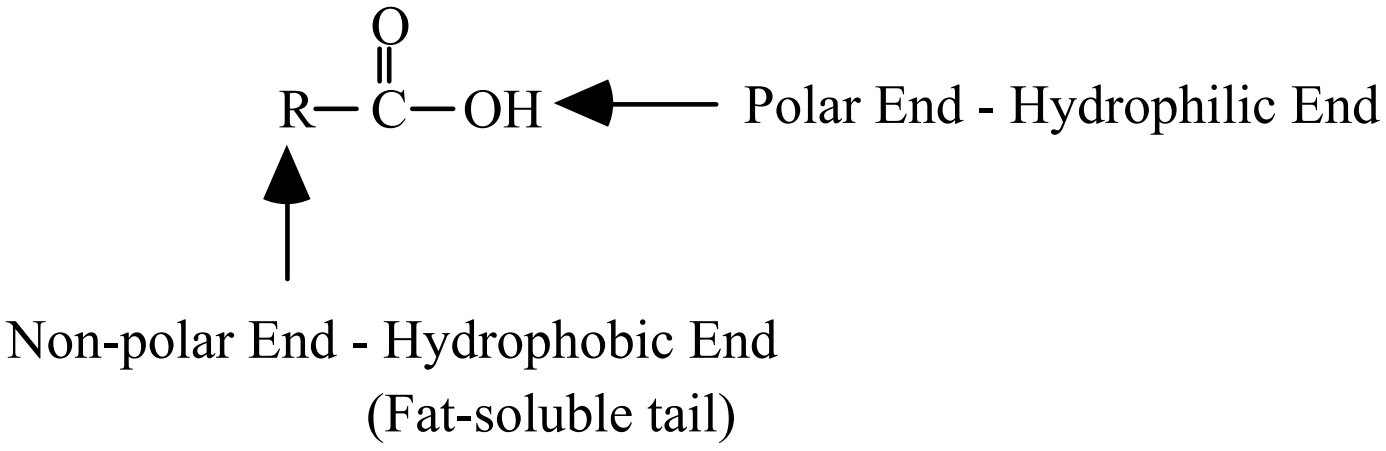
1. Fatty acids
2. Neutral fats and oils
3. Waxes
4. Phospholipid
5. Sterols
6. Fat soluble vitamins

Lipids in foods occur in three different forms:

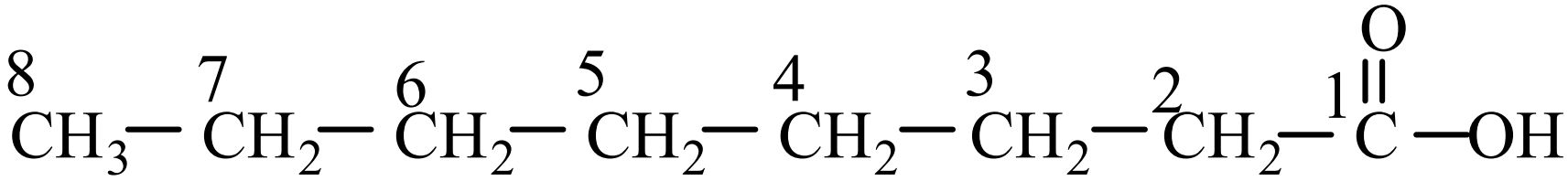
1- **Simple lipids-Fats,oils are triglycerides-They are the ester of fatty acids with glycerol(all edible fats and oils); waxes are esters of fatty acids with longer chain alcohols.**
2- **Composite lipids: Contain components other than lipids.Ex:Phospholipids,Lipoproteins,Glycolipids: These are found in bound form with other constituents; their analyses is harder than simple lipids, because you have to hydrolyse the molecule before analysis.**
3- **Derived lipids: These are substances derived from lipids and are soluble in lipid-extracting solvents(i.e. fat soluble pigments, sterols, antioxidants like tocopherols and Vitamins A,D, E,K (fat-soluble vitamins).They are not true lipids but are found in lipid phase. They behave like lipids. In most analyses you will get them in the lipid fraction.**

Fatty Acids


O
R—C—OH
#1 Carbon
Acid Group

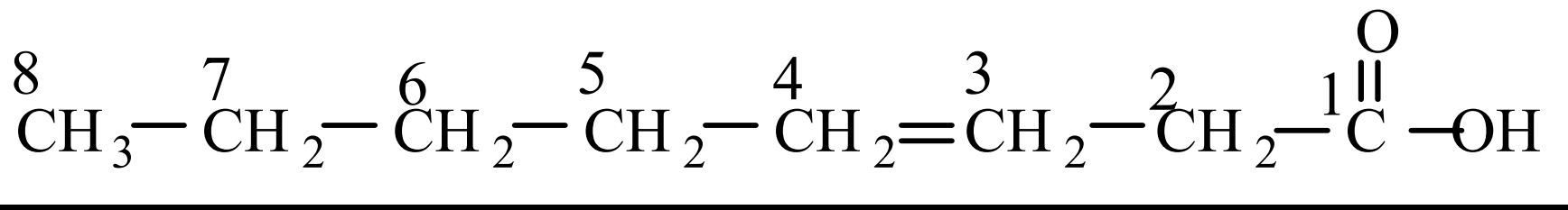

O
R—C—OH
Polar End - Hydrophilic End
Non-polar End - Hydrophobic End
(Fat-soluble tail)

Saturated Fatty Acids

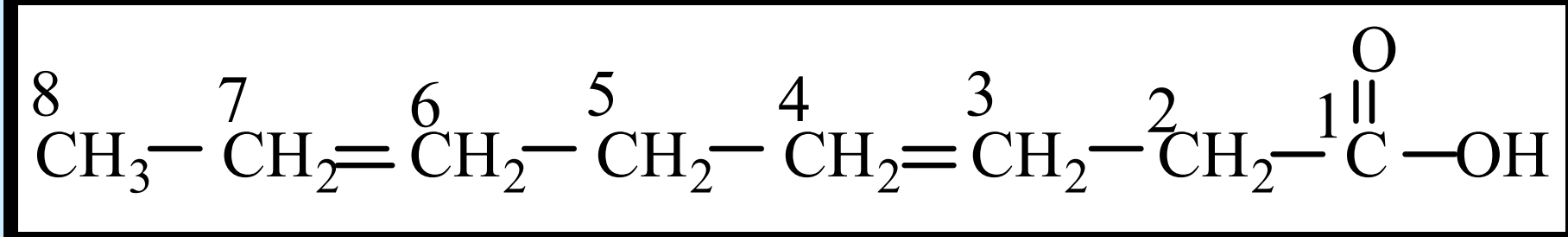


Octanoic Acid

Unsaturated Fatty Acids



3 - Octenoic Acid

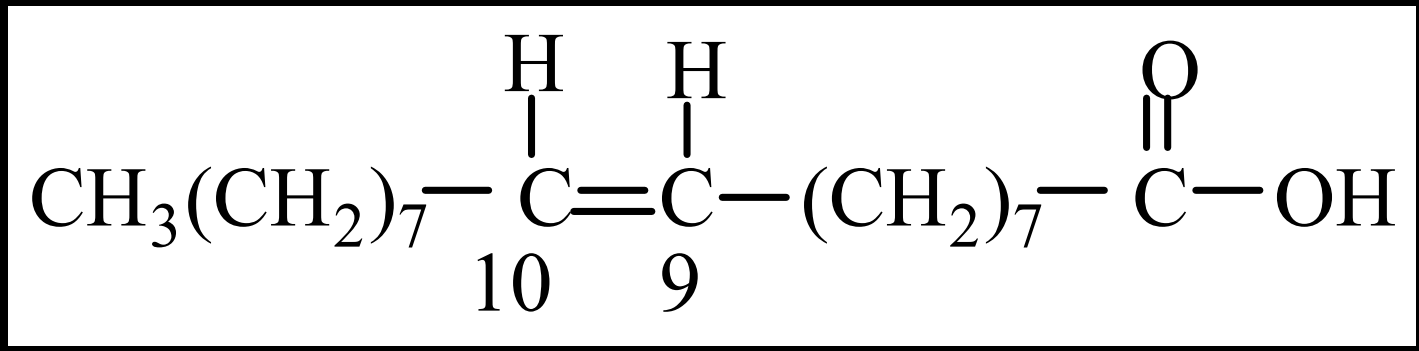


3, 6 - Octadienoic Acid

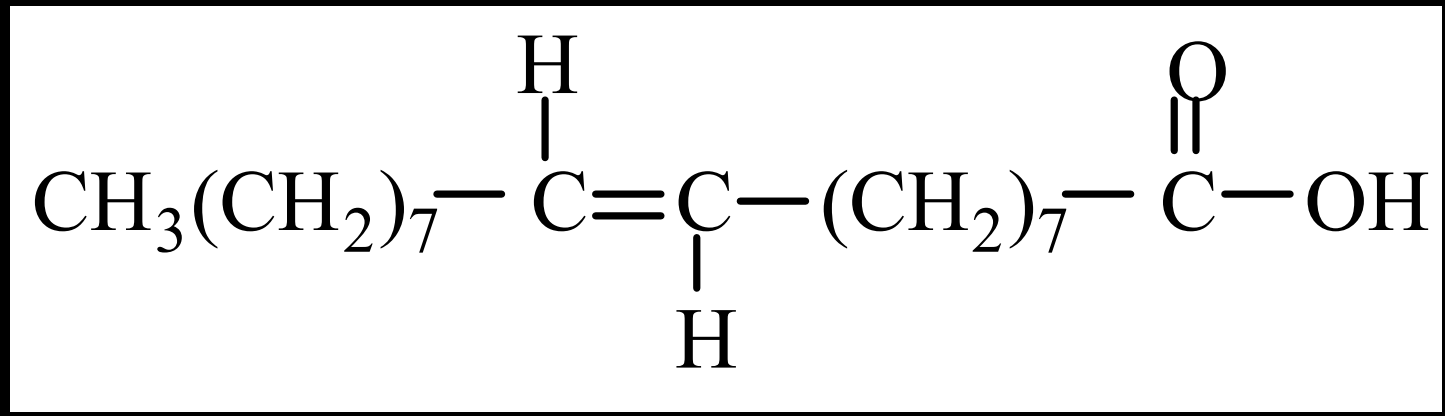
Short hand: 8:1 (Δ3)

8:2 (Δ3,6)

Cis And Trans Fatty Acids



Cis 9 - Octadecenoic Acid (oleic)



Trans 9 - Octadecenoic Acid (elaidic acid)

Polyunsaturated Fatty Acids

Linoleic acid:	Cis, cis, 9, 12 - Octadecadienoic acid
Linolenic acid:	Cis, cis, cis 9, 12, 15 - Octadecatrienoic acid
Arachidonic acid:	Cis, cis, cis, cis 5, 8, 11, 14 - Eicosatetraenoic acid

Linoleic Acid

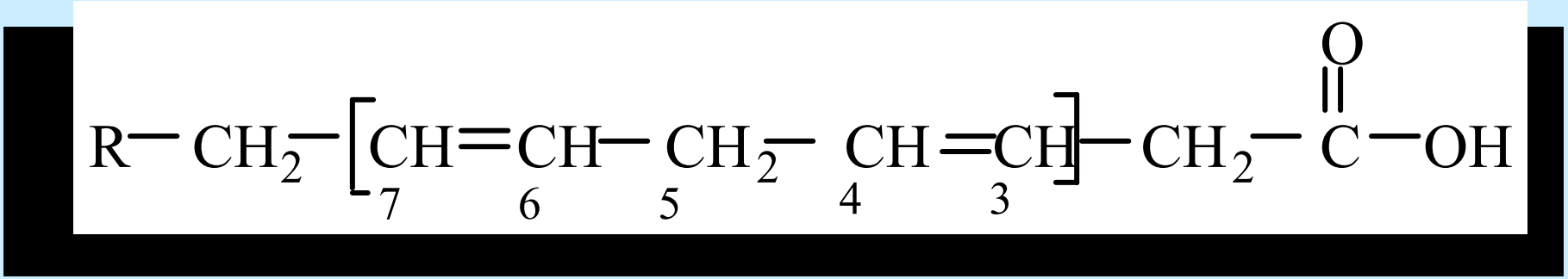
↓

Linolenic Acid

↓

Arachidonic Acid

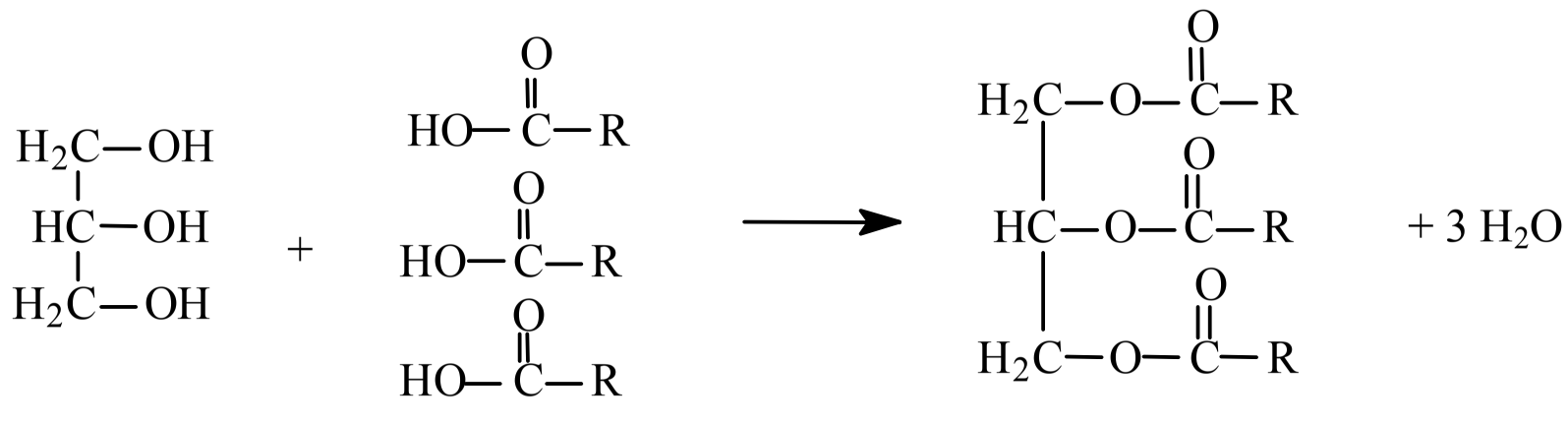
Naturally-occurring fatty acids



1. Cis form
2. Not conjugated --- isolated double bond.
3. Even numbered fatty acids.

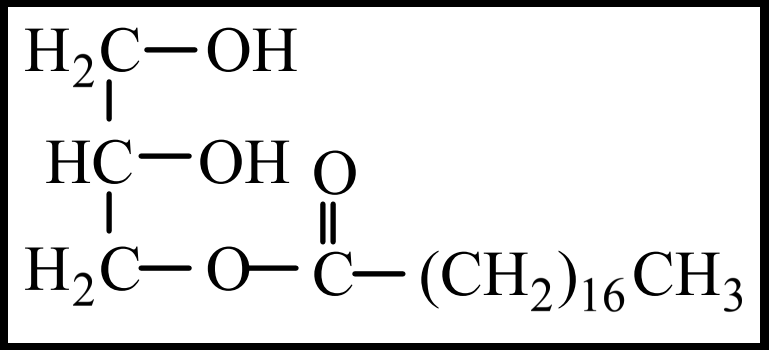
FAT AND OILS

Mostly Triglycerides:

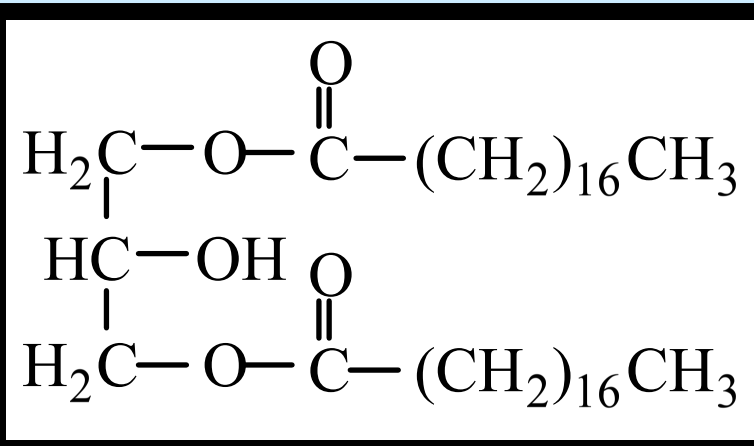


Glycerol 3 Fatty Acids

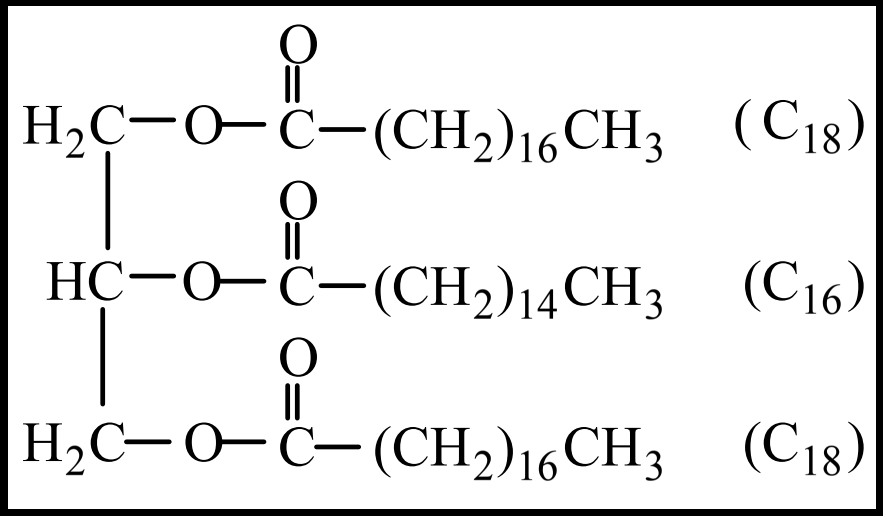
GLYCERIDES



Monoglyceride (α - monostearin)



Diglyceride (α, α' - distearin)



Triglyceride (β - palmityl distearin)

α - oleodipalmitin

1 - oleodipalmitin

α - Linoleyldiolein

1 - Linoleyldiolein

FATS AND OILS ARE PRIMARILY TRIGLYCERIDES (97-99%)

Vegetable oil - 68% of world supply

- Cocoa butter - solid fat
- Oil seeds - liquid oil

Animal fat - 28% (from Hogs and Cattle)

Marine oil - 4%

- Whale oil
- cod liver oil

Fatty Acid Compositions (%) of Fats and Oils

Fatty Acids	Butter	Coconut	Cottonseed	Soybean
4	3			
6	3			
8	2	6		
10	3	6		
12	3	44		
14	10	18	1	
16	26	11	4	12
16:1	7		1	
18:0	15	6	3	2
18:1	29	7	18	24
18:2	2	2	53	54
18:3	2			8

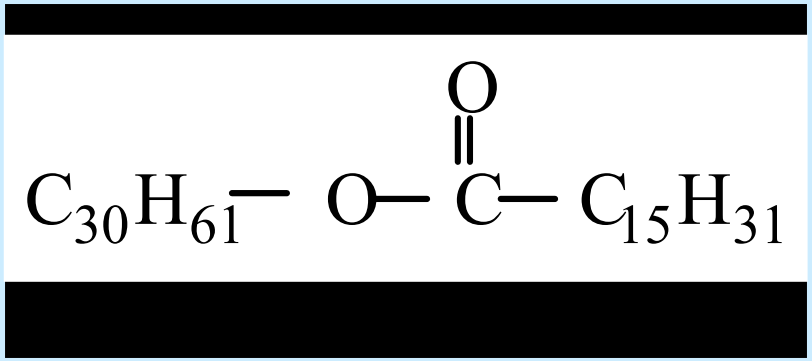
WAXES

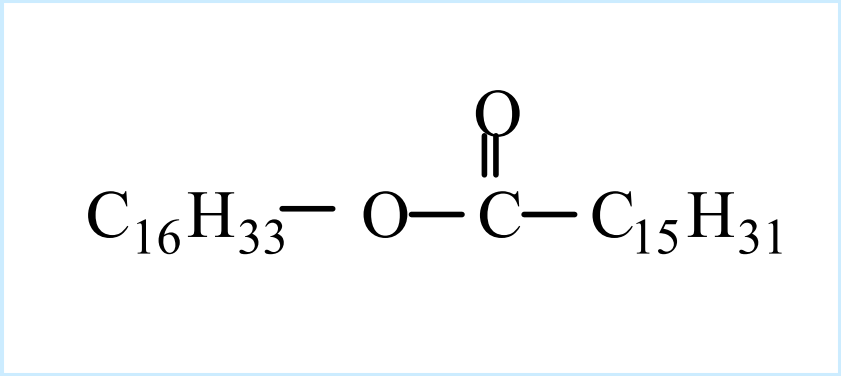
Fatty acids + Long chain alcohol

Important in fruits:

1. Natural protective layer in fruits, vegetables, etc.
2. Added in some cases for appearance and protection.

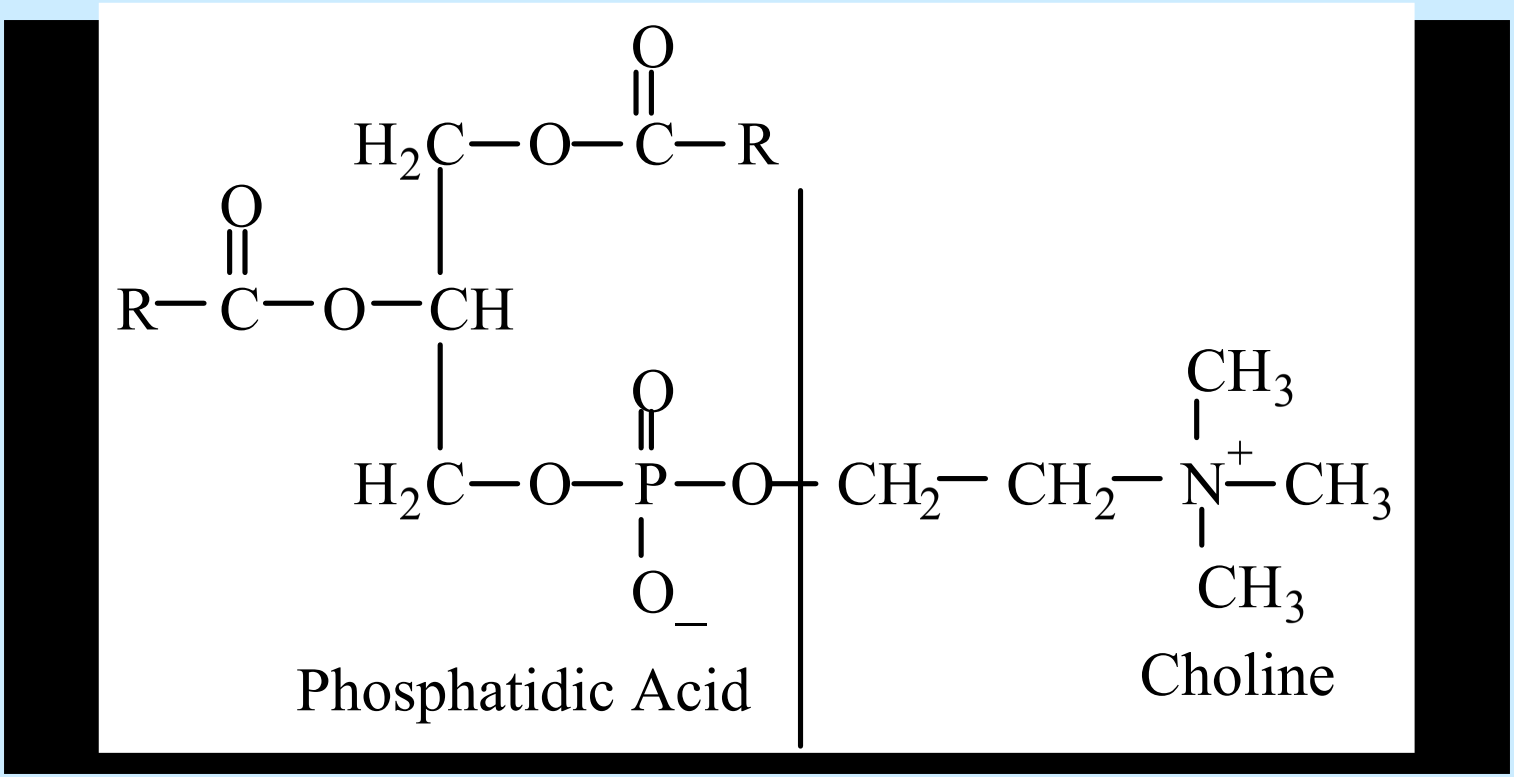
Beeswax (myricyl palmitate), Spermaceti (cetyl palmitate)





PHOSPHOLIPID

Lecithin (phosphatidyl choline)



STEROLS

Male & female sex hormones

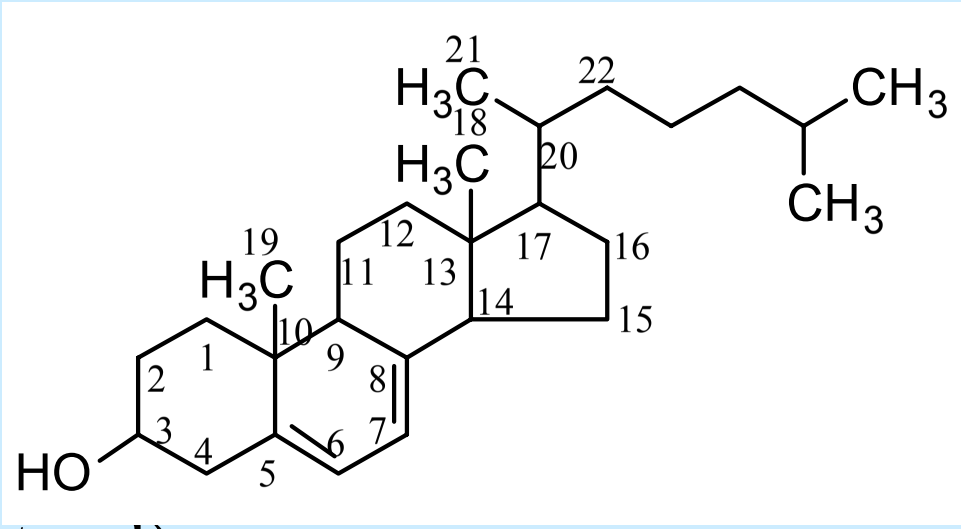
Bile acids

Vitamin D

Adrenal corticosteroids

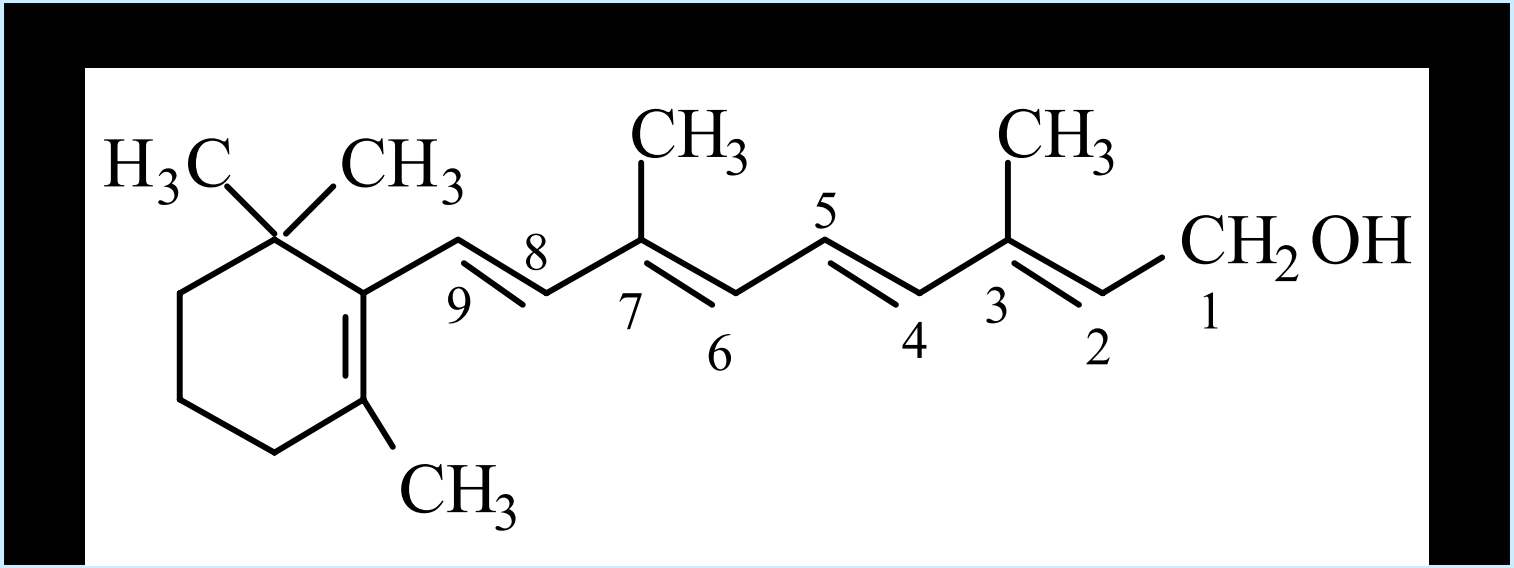
Cholesterol

Phytosterols(i.e. B-sitosterol)

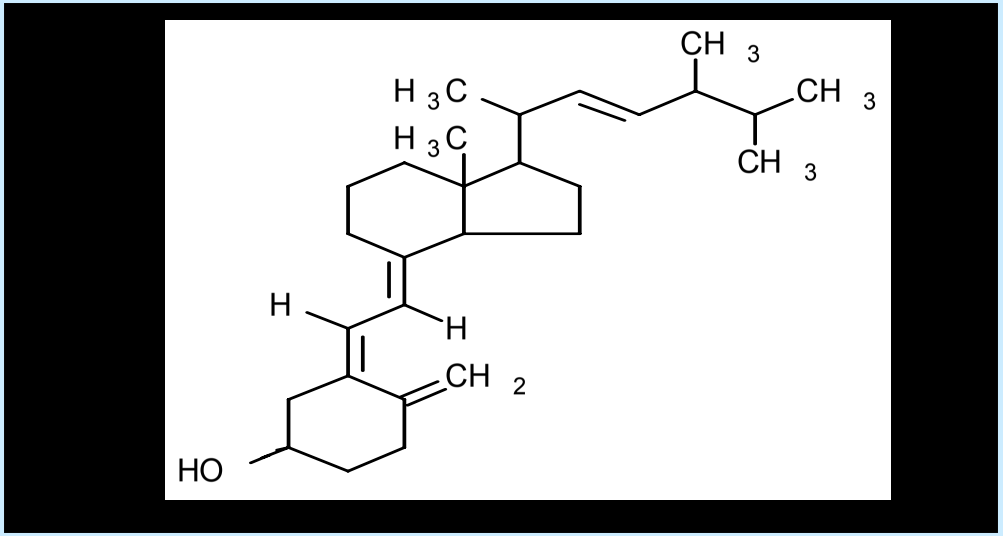


FAT SOLUBLE VITAMINS

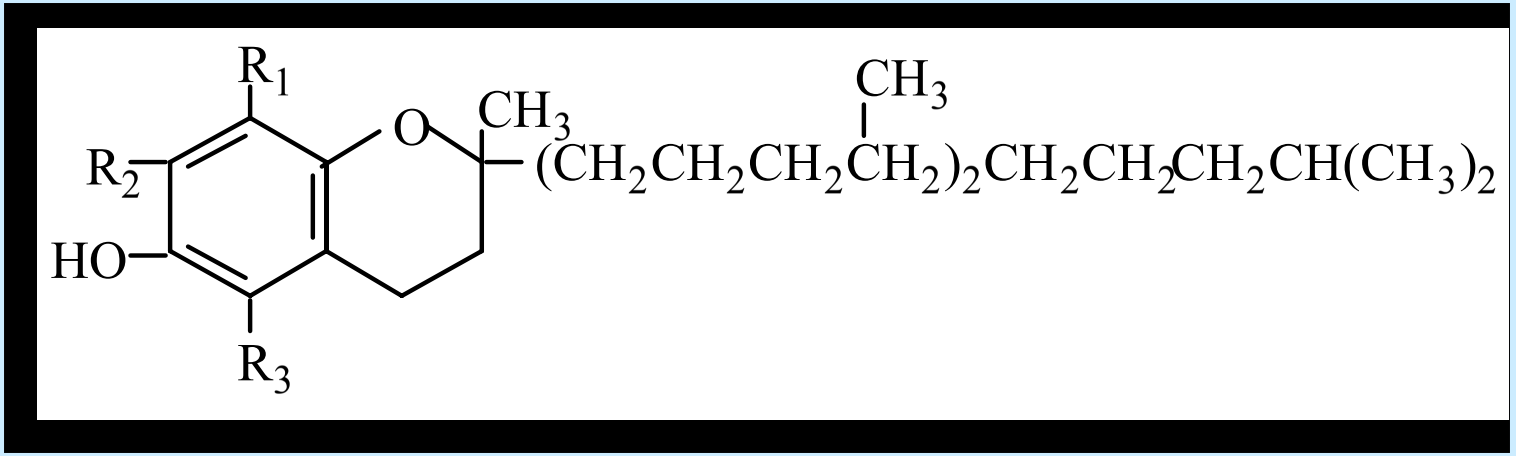
Vitamin A:



Vitamin D2:



Vitamin E:



Fat contents of selected foods:

Liquid vegetable oils ≅ 100%

Margarine and butter ≅ 80% → they are emulsions of lipid in water

Almonds :%55, Walnuts:%65, Cereals: 3-5%

Sunflower seeds, hulled : ~60%

Chocolate: ~35% , Milk : 37%, Milk powder : 27.5%

Tuna fish : 4% (but there are some fatty fish like anchovies containing 15% fat)

Ia. General procedure for solvent extractions:

1. **Sample Preparation: Plant or animal tissues should be homogenized by grinding,blending etc, since particle size is important; it has to be ideal particle size for maximum extraction yields(i.e.0.4mmϕ). then it has to be dried before extraction, because if any moisture is let stay in the samples, it interferes with extraction, and the solvent's extraction capability decreases.Also hydrolysis is needed to break down any bound lipids, either by acids(HCl or H_2SO4) or by enzymes like Takadiastase.**

2. **Solvent selection: You need different types of solvents for extracting different types of lipids:**

(Chloroform+methanol) is ideal for lipoproteins, whereas petroleum ether fractions(C6 and C5) are ideal for true lipids (triyglycerides), and ethyl ether for extracting oxidized lipids. Sometimes a ternary mixture works better (CHCl3: methanol:H2O) for extraction from animal tissues; water-saturated butanol for cereal lipids.

LIPID CONTENT ANALYSES

1. Gravimetric Methods

   (1) Wet extraction - Roese Gottliegb & Mojonnier.

   (2) Dry extraction - Soxhlet Method.

2. Volumetric Methods (Babcock, Gerber Methods)

1. Gravimetric Methods

(1) Wet Extraction - Roese Gottlieb & Mojonnier.

For Milk:

1) 10 g milk + 1.25 ml NH_4OH mix. solubilizes protein and neutralizes.

2) + 10 ml EtOH - shake. Begins extraction, prevents gelation of proteins.

3) + 25 ml Et2O - shake and mix.

4) + 25 ml petroleum ether, mix and shake.

(2) Dry Extraction - Soxhlet Method.

Sample in thimble is continuously extracted with ether using Soxhlet condenser. After extraction, direct measurement of fat

- evaporate ether and weigh the flask.

Indirect measurement - dry thimble and weigh thimble and sample.

2. Volumetric Methods (Babcock, Gerber Methods)

Theory:

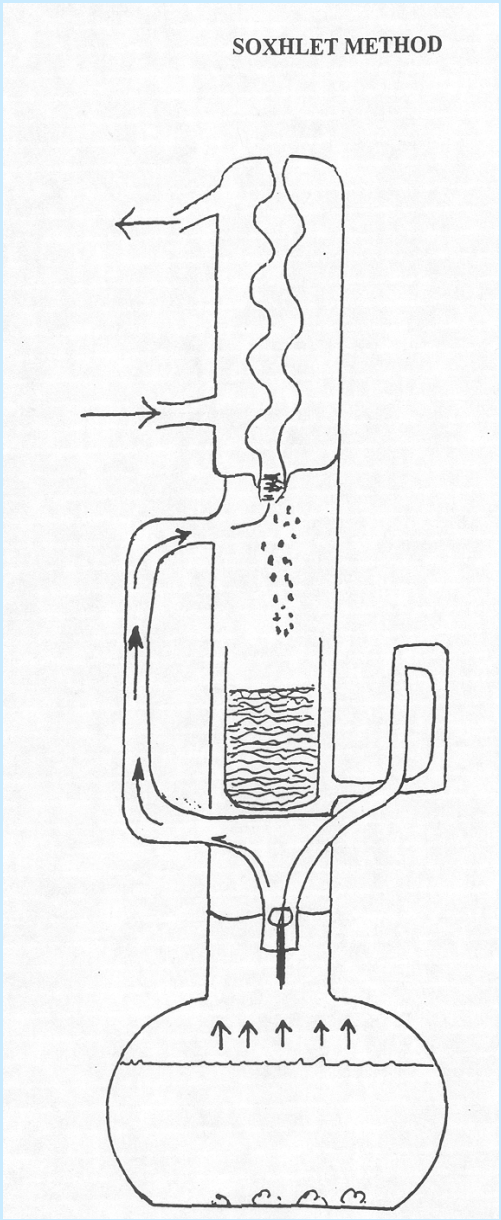
1. Treat sample with H_2SO_4 or detergent.
2. Centrifuge to separate fat layer.
3. Measure the fat content using specially calibrated bottles.

Methods:

1. Known weight sample.
2. H_2SO_4 - digest protein, liquefy fat.
3. Add H_2O so that fat will be in graduated part of bottle.
4. centrifuge to separate fat from other materials completely.

Types of Extraction:

a1.. **"Dry Extractions":** These are basically solid-liquid type of extractions,and the final results are gravimetric. They may be either batch, multiple-batch, continuous or countercurrent extractions. There are also automated processes,like FOSSLET extractors, where the predetermined number of stages is (4-5), and cartridges are used to contain the sample. In complex matrices, digestion may be required to liberate bound lipids by hydrolysis before starting the extraction.



Solvent Extraction Methods:

a.1.1.Continuous(Goldfish)or semicontinuous(Soxhlet) or discontinuous(Majonnier) solvent extraction methods: Solvent from a boiling flask continuously flows over the sample held in a thimble.Fat content is measured by weight of fat removed.

a.1.2.Elevated pressure/Temperature methods: SFE(Supercritical fluid extractions):ince it avoids the use of hazardous organic solvents, and uses inert, inexpensive, and nontoxic gases like CO_2, it is gaining more popularity.

a.2. "Wet nonsolvent extractions" : are preferred for liquid samples, since they involve liq-liq extraction (partitioning of material between two immiscible liquids) and yield volumetric results.
Ex: Gerber and Babcock type of extraction of milk are examples of wet extraction. In milk, the fat globules are present in the form of an emulsion of "oil in water", surrounded by a thin film of protein which has to be removed or broken by acid treatment before separating fat from water phase.
Gerber Procedure: 11 ml milk and 1 ml isoamyl alcohol are mixed to improve phase separation, by increasing the water phase.Then H_2SO4 (Sp. gr.:1.82 for breaking down the lipid-protein barrier) is added and mixture is centrifuged for 5 min, submerged in H_2O bath (65°C, 5 min). Volumes of two respective phases gives fat-water ratio, or the upper layer gives fat content.

I.b. Indirect Methods for Quantification of Lipids

Especially geneticists in plant breeding research studies require "non-destructive" types of lipid analyses. For this purpose, many "instrumental systems" are developed:

*NMR:Low resoolution nuclear magnetic resonance:The measured NMR value is related to the total number of protons in oil fraction of seed , independent of protons in non-oil fraction, since protons have a different magnetic resonance in oil and in water phase. It gives very good approximation of lipid levels. Calibration tables or curves are used for different H 2 0 levels(fig. 13-8).

*X-ray absorbtion: Is an approved method for meat carcass samples. Lean (yağsız) meat absorbs more x-ray than fatty tissues (carcass-lipids)

Conductance: Lean meat is 20-fold better conductor for electrical current than fat.

Densitometric Methods: Density of seeds (i.e. Flaxseed) is proportional to its oil content. Likewise, density of extracting solvent changes proportional to the fat content it has extracted(i.e.perchloroethylene in FOSSLET). For meat , there is a method using a cylindrical container with a piston plate that holds meat under constant hydraulic pressure. Measured impression depth is related to fat content.

Refractometry: η_D of the mixture (bromonaphtalene and fat extracted by it from chocolate) is used for approximation of fat in chocolate.

II. Assays on Extracted Lipids
-FAT CHARACTERISATION(Ch.14):

II.a. **Purity Criteria**: For authenticity testing and determination of adulteration. Most analytical values of certain oils and fats are quite characteristic:

a.1. **Physical purity criteria** are refractive index, melting point, colour, density,. By measuring these criteria, we will find values within certain accepted limits and ranges. But these alone are not enough for authenticity characterisation.

Color:Either Lovibond color scale using standard red-yellow-blue filters, or determination of "photometric color index-PCI)* is required for objective description of color.

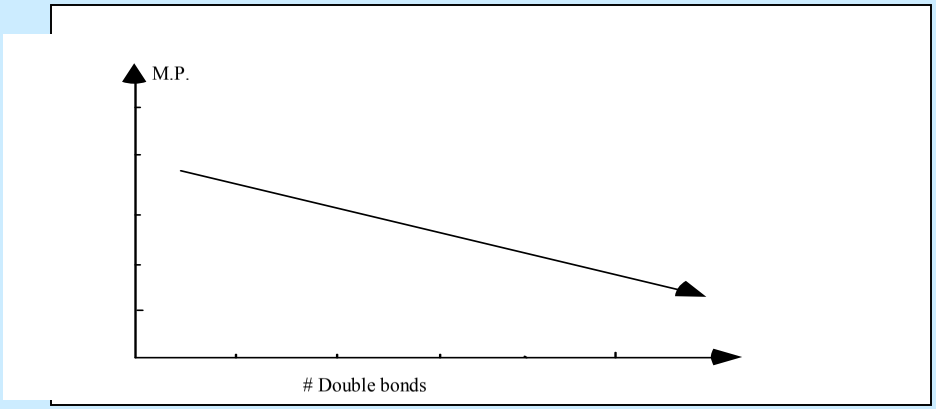
$$* PCI = 1.29(A_{460}) + 69.7(A_{550}) + 41.2(A_{620}) - 56.4(A_{670})$$

Refractive Index:RI may also be used for controlling hydrogenation, since RI will decrease linearly as unsaturation decreases.

Melting point, smoke, flash and fire points

Effects of Double Bonds on the Melting Points

F. A.	M. P. (^{0}C)
16:0	60
16:1	1
18:0	63
18:1	16
18:2	-5
18:3	-11
20:0	75
20:4	-50



a.2. **Chemical purity criteria:** These are very characteristic for each oil and fat types:

- Saponification value measuring average f.a. molecular weight and chain length
- Iodine value measuring level of unsaturation

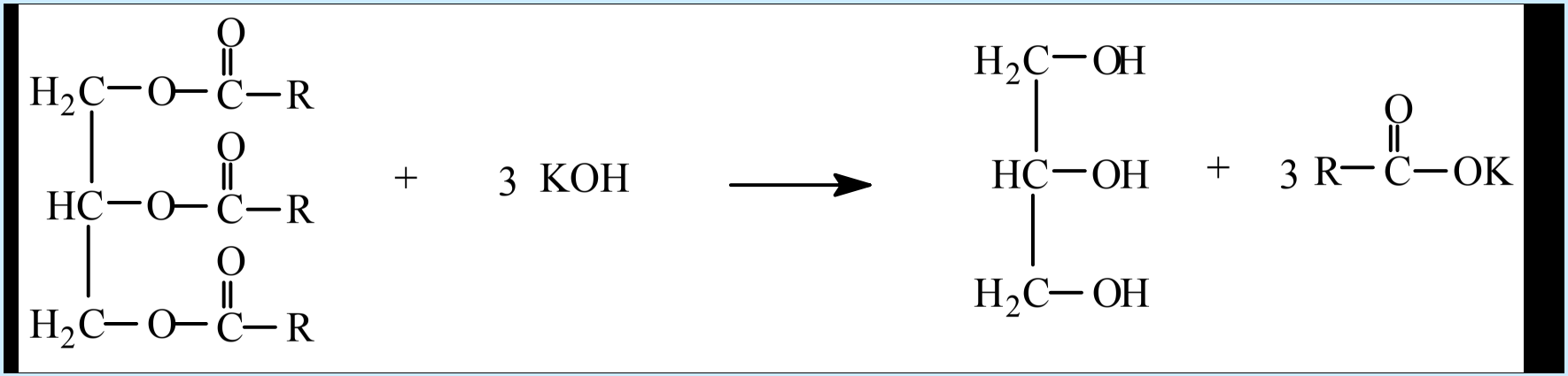
- Fatty acid composition(FAME),

- Individual sterol and tocopherol compositions.

With an integrated evaluation of results of purity criteria analyses, we can be sure of identity and source of fats and oils (Reference methods in AOCS,IUPAC and DGF).

1. Saponification Value(14.3.9)

Saponification - hydrolysis of ester linkages under alkaline (KOH)condition.



Saponification Value of Fats and Oils

Fat	Saponification Value
Milk Fat	210-233
Coconut Oil	250-264
Cotton Seed Oil	189-198
Soybean Oil	189-195
Lard	190-202

Saponification Value Determination

Saponification Value=-mgs of KOH required to saponify 1 g of fat.

1. **5 g in 250 ml Erlenmeyer.**
2. **50 ml KOH in Erlenmeyer.**
3. **Boil for saponification.**
4. **Titrate with HCl using phenolphthalein.**
5. **Conduct blank determination.**

$$SP\# = \frac{56.1(B - S) \times N \text{ of HCl}}{\text{Gram of Sample}}$$

B - ml of HCl required by Blank.

S - ml of HCl required by Sample.

SV is an index of the mean molecular weight of the triacylglycerols in sample oil or fat.

The mean molecular weight of triacylglycerols when divided by three will give an approximate mean molecular weight of fatty acids present.

2. Iodine Number (14.3.8)

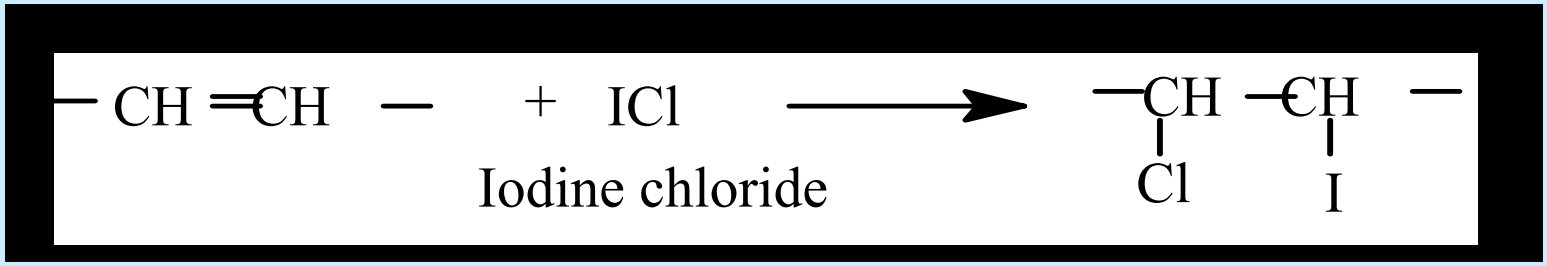
Iodine value (IV): Halogens add to double bonds.
IV is the number of grams of iodine that adds to 100 gram of oil.
If 1 g of the fat adsorbs 1.5 g of iodine, then IV = 150.

It is a measure of saturation-unsaturation.

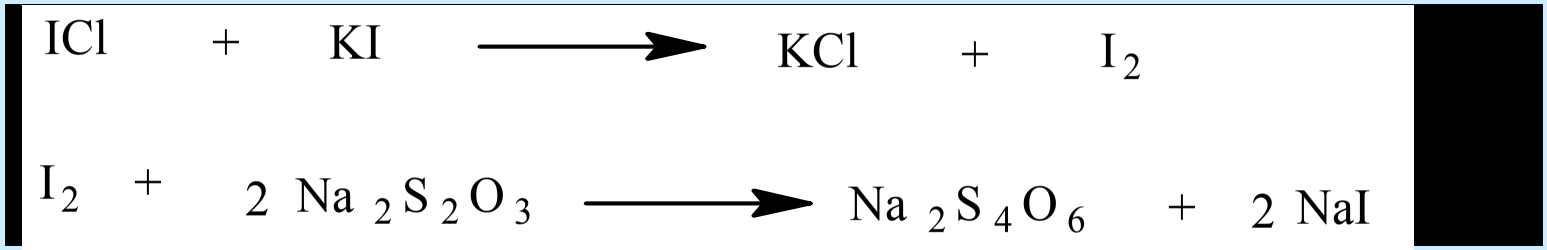
Iodine Value Determination

Iodine Value = (ml of $Na_2S_2O_3$ volume for blank - ml of $Na_2S_2O_3$ volume for sample) × N of $Na_2S_2O_3$ × 0.127g/meq × 100

Weight of Sample (g)



Excess unreacted ICl



Iodine Numbers of Selected F.A.

Fatty Acids	# of Double-bonds	Iodine #
Palmitoleic Acid	1	95
Oleic Acid	1	86
Linoleic Acid	2	173
Linolenic Acid	3	261
Arachidonic Acid	4	320

If molecular weight and iodine number of f.a. is known, we can calculate the number of double bonds:IV/MW

During oxidation, there will be a decline in unsatuartion and therefore in IV:

The ranges of Iodine Value for olive oil, which is an index of its degree of unsaturation, is 75-94 in the Codex. For Italy this value fluctuates between 79 and 88, but for Tunisia the range is 80-97. There may be such regional or climatic variations, therefore, even though results are quite meaningful, sometimes additional tests are required for final confirmation.

GC Analysis for Fatty Acids (14.6.1)

1. Extract fat.

2. Saponify (hydrolysis under basic condition).

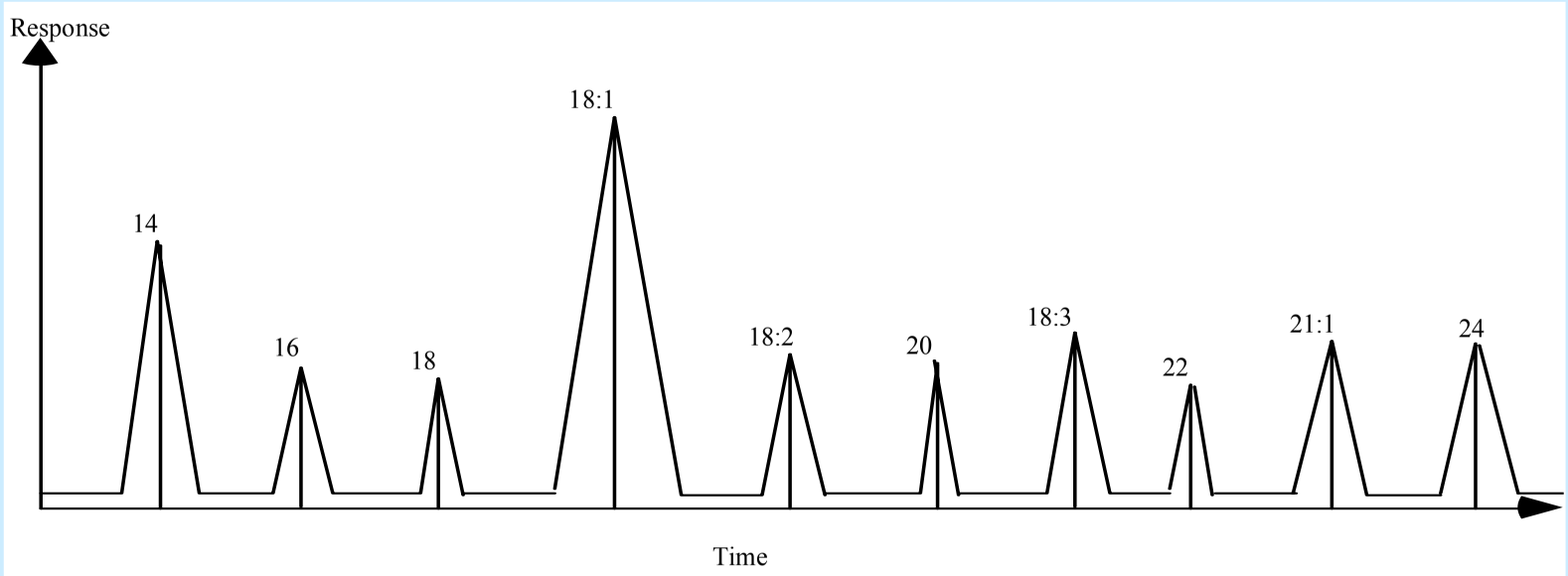
3. Prepare “fatty acid methyl esters”or FAME (by Sodium methoxide - CH3ONa or BF3-methanol reagent).

4. Chromatography of methyl esters.

5. Determine peak areas of fatty acids.

   (Fatty acids are identified by their specific retention times).

6. Compare with response curve of standard.



For analysing FAME:
The FAME are carried in and out of the GC Column by the carrier gas and each will leave the column only after a specific retention time,Rt, which is dependant on the chain length and degree of saturation of the fatty acid. Generally, as carbon number as well as double bonds in f.atty acids increase, the retention time Rt will also increase.

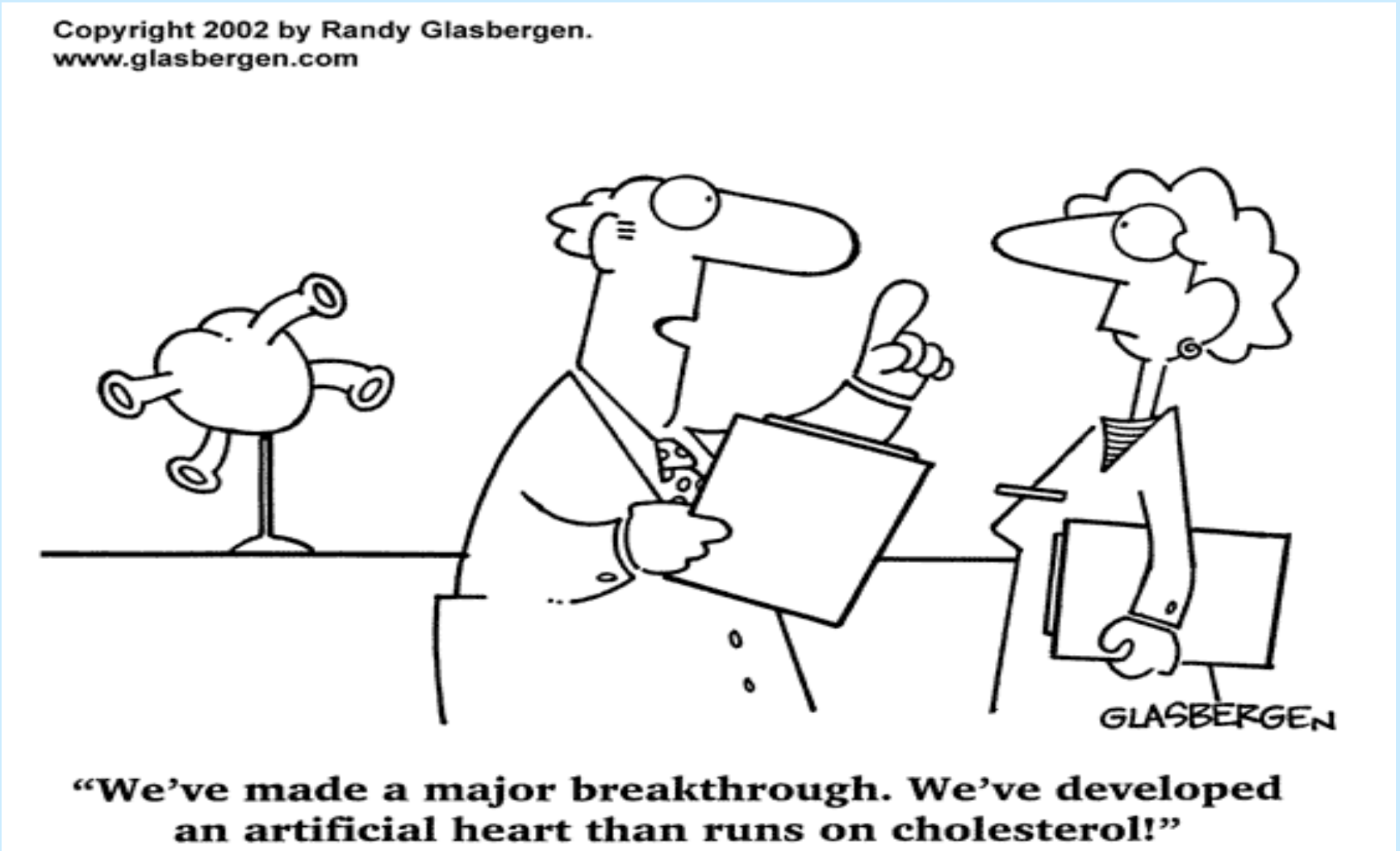
FAME: The fatty acid composition determined by GC (gas chromotography) is a real "Fingerprint" .
It also serves for "nutritional labelling": saturateds,monounsaturates, polyunsaturates, trans f.a. etc.

For authenticity testing of butters, this analysis by itself may not sometimes be sufficient, since some margarines might have similar composition of FAME with butters.

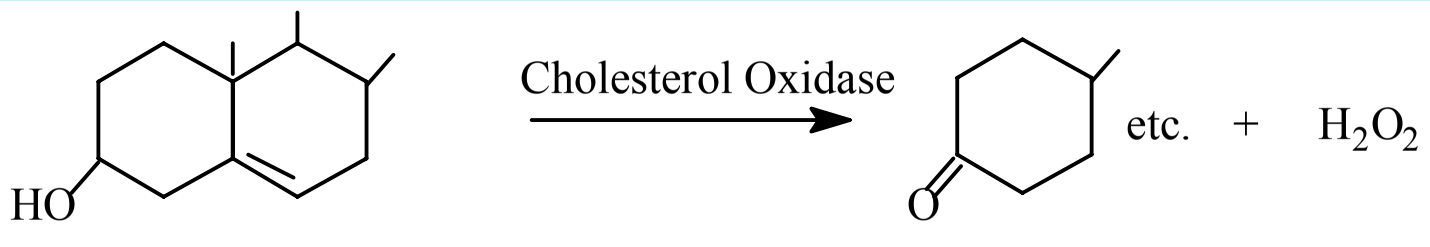
In this case,the respective sterol composition should also be determined. Sterol analysis is done by TLC followed by GC of the unsaponifiable fraction of lipids. (There are "Phytosterols" - stigmasterol, campesterol, β-sitosterol- in the vegetable sterol fraction, but only "cholesterol" in animal fats).

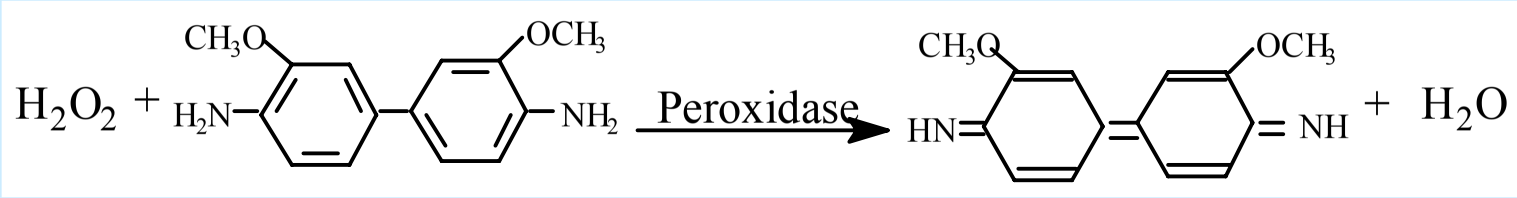
CHOLESTEROL DETERMINATION(14.6.4)

Cholesterol by GLC:saponify extracted lipid,derivatize ⟶ GC



Enzymatic Determination: Cholesterol Oxidase

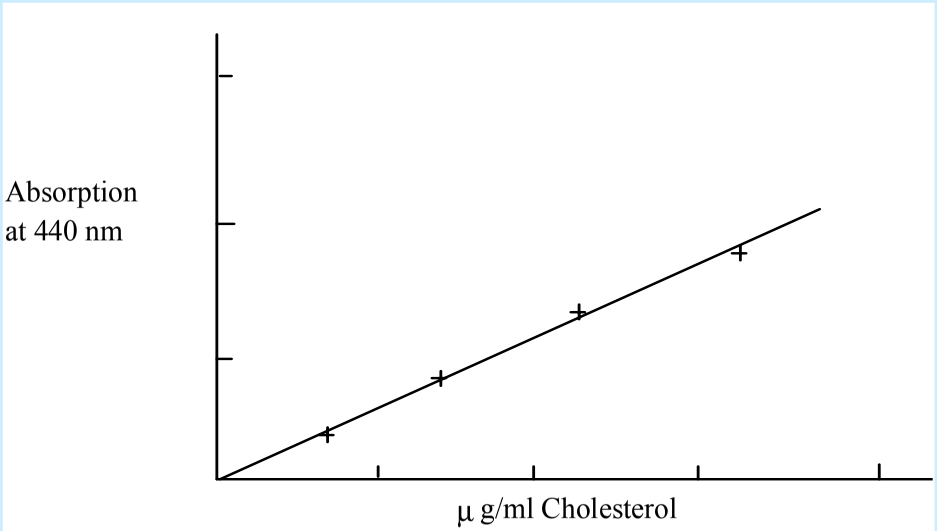




0-Dianisidine (Colorless)

Oxidized 0-Dianisidine (Brown color)At 440 nm

Spectromertic Absorption Standard Curve of Cholesterol

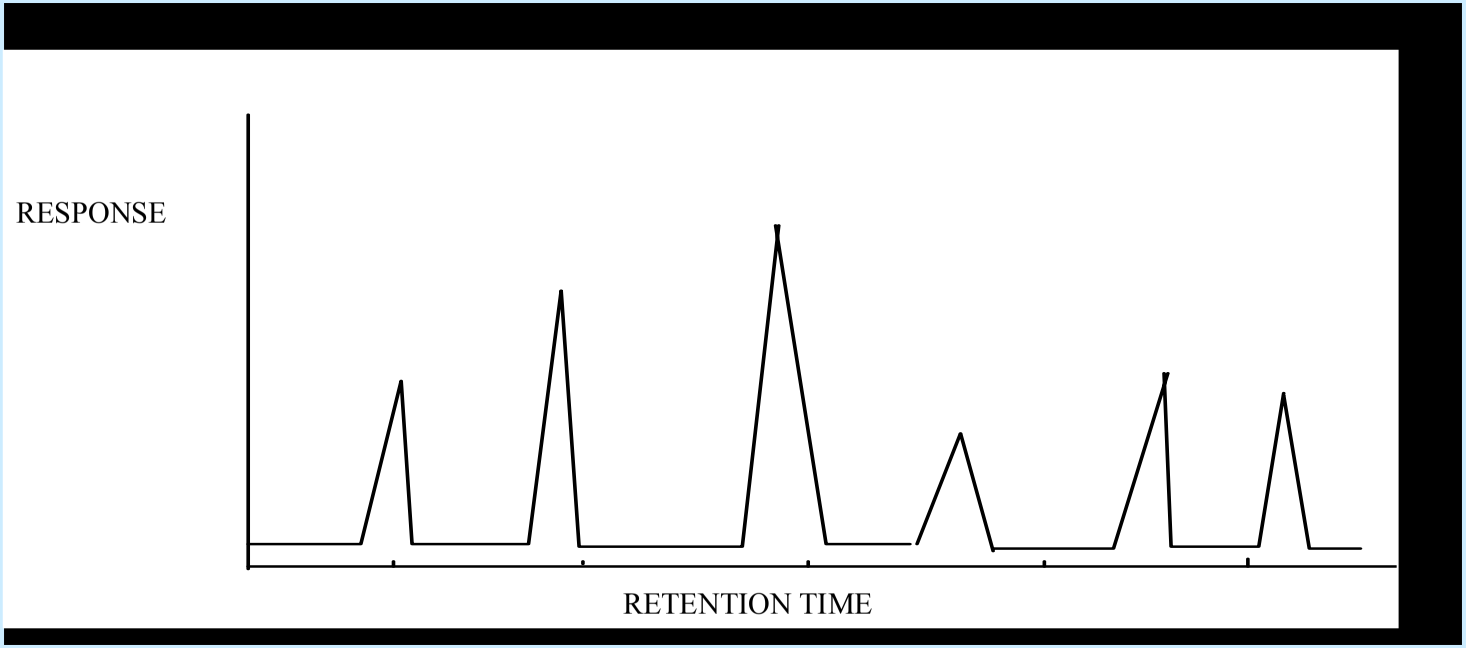


TRIGLYCERIDE ANALYSIS BY LIQUID CHROMATOGRAPHY

Soybean Oil

Solvent CH_3CN/HF

Column 84346 (Waters Associates)



Oleate-containing triglycerides in olive oil

Fatty Acid Composition	Total Acyl Carbons: Unsaturation	Equivalent Carbon Number
OL2	54:5	44
O2L	54:4	46
OPL	52:3	46
O3	54:3	48
OSL	54:3	48
O2P	52:2	48
O2S	54:2	50
OPS	52:1	50
OS2	54:1	52

Further Specific Identification Tests for SomeFats and Oils:

(For detection of adulteration)

For butter: Reichert-Meissel index for butter is a measure of its content of volatile and water-soluble butyric acid; >24.

For olive oil: Squalene (7000 mg/kg) is a very specific constituent of unsaponifiable part of olive oil, whereas in others, it varies between 30-400ppm.

For cottonseed oil: Halphen test for detecting presence of cyclopropenoic acids which form a red color on heating with amyl alcohol and CS2.

For sesameseed oil: Villavechia test: unsaponifiables of sesameseed oil only will form pink color with furfural.

For cocobutter: Triglycerides C50 and C54 are dominant in cocobutter but not in CBE and CBS.

II b. Quality Criteria

IIb.1. M I U (Moisture+Insoluble Impurities) (dirt, oil seed particles in crude oils and soap in refined oils)+(unsaponifiables) : They are nonfatty constituents of oils which increase refining costs for crude oils . Therefore, every percent of increase in MIU content brings parallel price decreases in crude oil purchases. Also soap>5 ppm left in refined oils will be detrimental to oil quality .

IIb.2 Fat rancidity and stability

Rancidity: "Development of objectionable flavours and odours resulting from lipolysis or lipid break-down (either due to hydrolytic or to oxidative reactions)leading to lower consumer acceptance".

Stability: "Capability to remain fresh or maintain fresh taste+odour" or "resistance to spoilage during storage or "resistance of lipids to rancidity"

RANCIDITY(Hydrolytic and Oxidative)

A-Hydrolytic Rancidity: Involves chemical or enzymatic(lipase from microorganisms or plant cells) hydrolysis of fats into free fatty acids and mono or di-glycerides. Relevant Tests:AV and ffa

a. A.V.=The number of miligrams of KOH required to neutralize 1 gram of fat or oil.

T and aw ↑

$$AV = \frac{\text{ml of KOH x N x 56}}{\text{Weight of Sample}} = \text{mg of KOH}$$

b. FFA: Free fatty acids= here, acidity is expressed as percentage of weight of a specific free fatty acid in a fat or oil,thus taking into consideration the M.W. of the individual f.a.:

* In olive oil, expressed as oleic acid, MW = 182
*In palm oil, expressed as palmitic acid,MW = 256

$$FFA \quad \% = \frac{V \ x \ MW \quad fatty \quad acids}{Weight \quad (1000 \quad)} x \ 100$$

* %FFA oleic acid= 0.503 x Acid value
* Acid value= 1.99 x % FFA oleic acid

Significance of AV and ffa:

This value will estimate the amount of crude oil that will be lost during refining, so will mean higher operating costs for refining plants, and leads to price decreases in crude oil purchases.

In refined oils, high values will mean either poor refining or fat breakdown in storage or use. In cases where the liberated ffa are volatile , ffa will be a measure of hydrolytic rancidity.

For Virgin olive oils < 4 % oleic acid, and for refined olive oils < 0.3; but for shorter chain f.a. like butyric,capric and even lauric (C4- C12), having much lower flavour thresholds(as in chocolate), the product will be inedible after its ffa content reaches > 0.02 %.

B-Oxidative Rancidity

"LIPID OXIDATION "

Major flavor problems in food during storage are mainly due to the oxidation of lipid.

Lipid Oxidation - free radical reactions.

1. Initiation.
2. Propagation.
3. Termination.

Methods for Measuring Present Status of Lipid Oxidation:

-Peroxide number: The amount of iodine liberated($Na_2S_2O_3$ titration) from saturated KI solution by fat , expresssed in meq of peroxide O_2/kg of fat

(Lea value=expressed in millimoles of O_2).

<10 for edible oils; measures only transient primary oxidation products like peroxides and hydroperoxides.

-Thiobarbituric acid value (TBA): The pink color formed by secondary oxidation products like malonaldehydes with TBA reagent is measured at 523 nm.Correlates better with sensory analyses.

-Anisidine value: Another color reaction of aldehydes(alkenals and dienals-secondary products of oxidation) with *P*- anisidine.

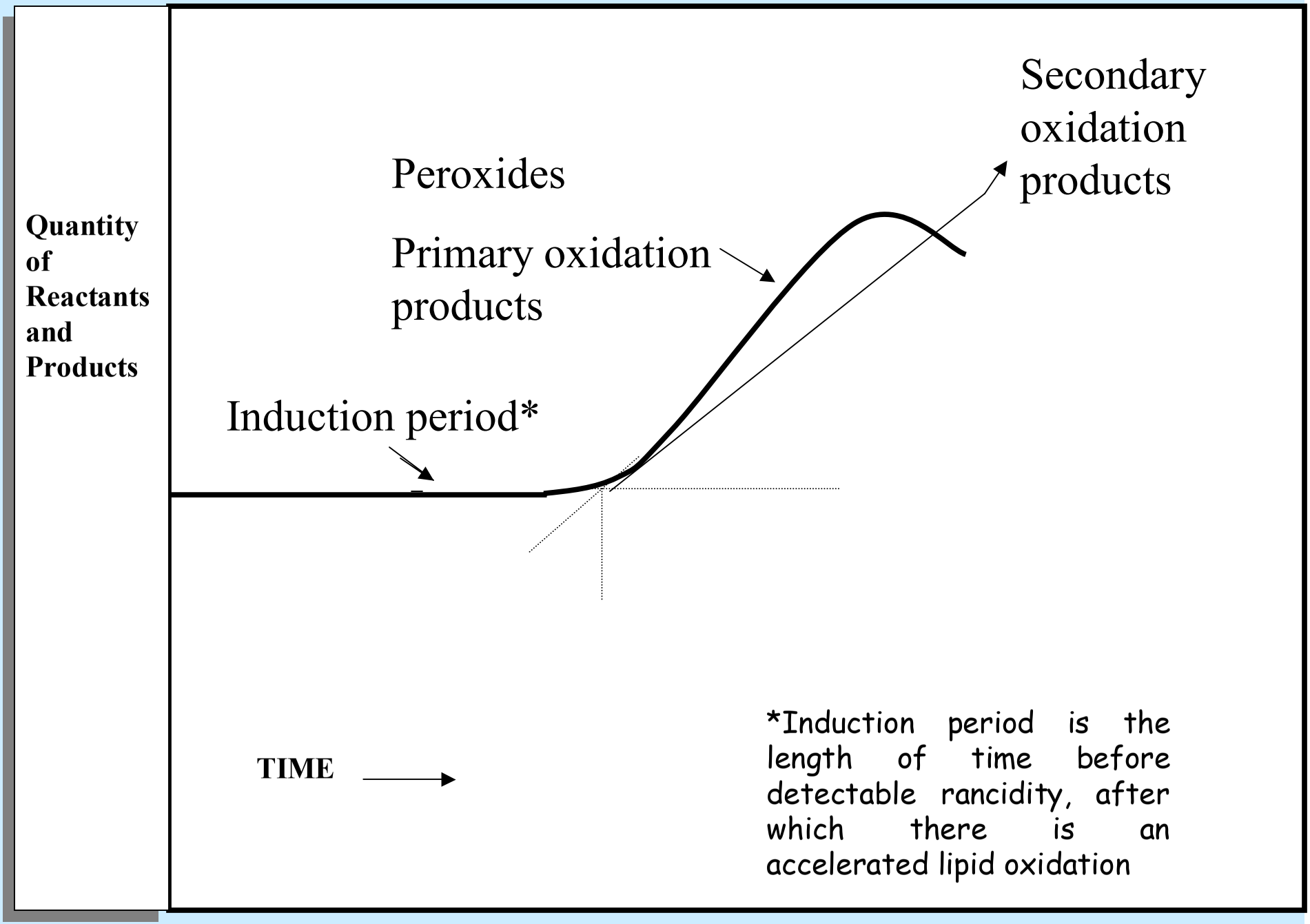
-Totox value : 2 times peroxide value + anisidine value = Total oxidation products (2 is because peroxides have 2 times more oxygen than aldehydes.)

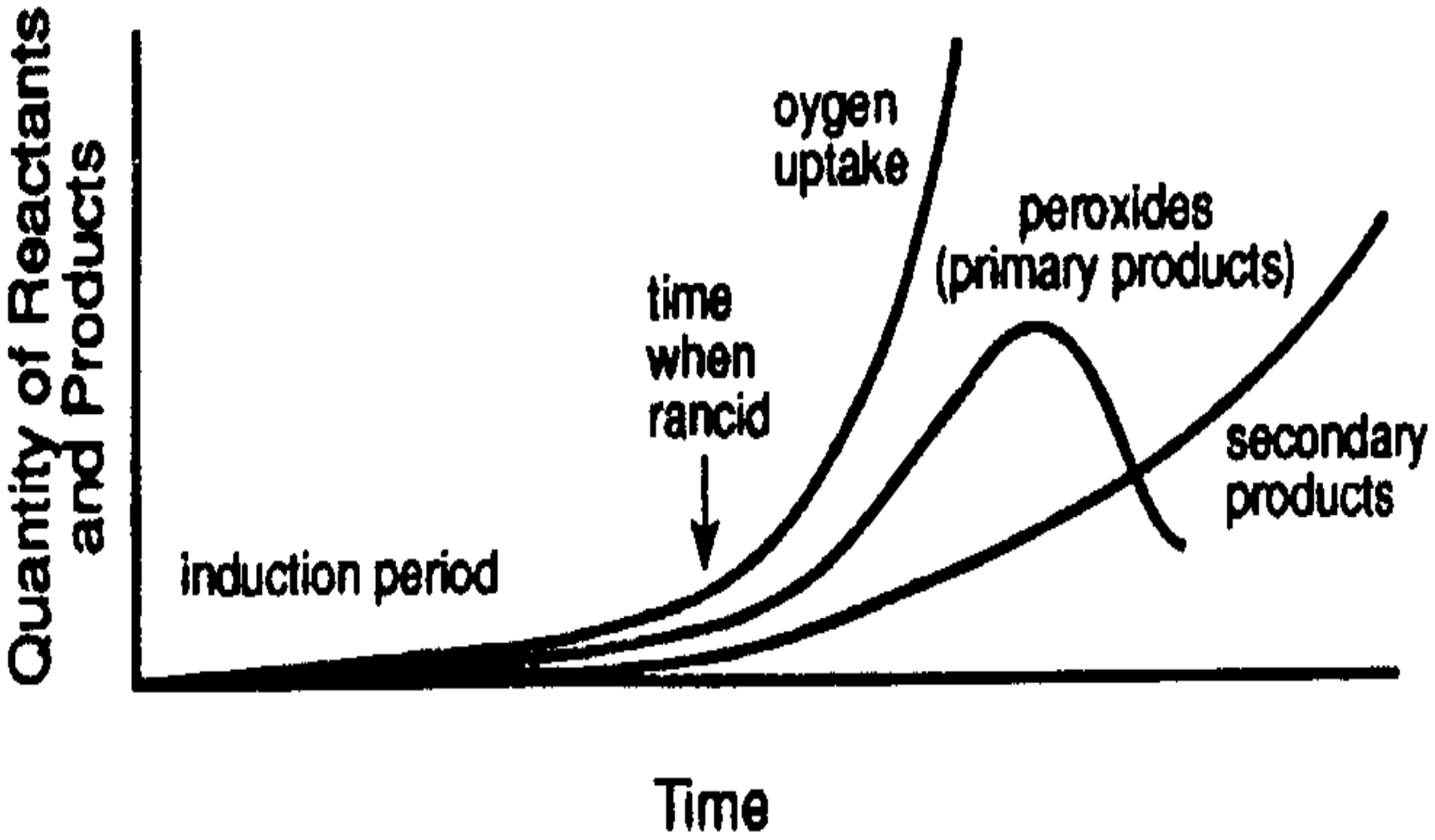
In most cases, rancidity is due to products of auto-oxidation of unsaturated fatty acids by progressive addition of oxygen to lipid components. Autoxidation reactions are catalysed by presence of prooxidants like Cu, Fe, sunlight and lipoxigenase enzyme.

The shelflife of fats and oils is assumed as the end of the induction period, after which the rate of formation of oxidation products (Peroxides, Oxides, Hydroperoxides) increases drastically.

IP is 1.3 hours for C18:3, 19 hours for C18:2, and 82 hours for C18:1 at 40°C.

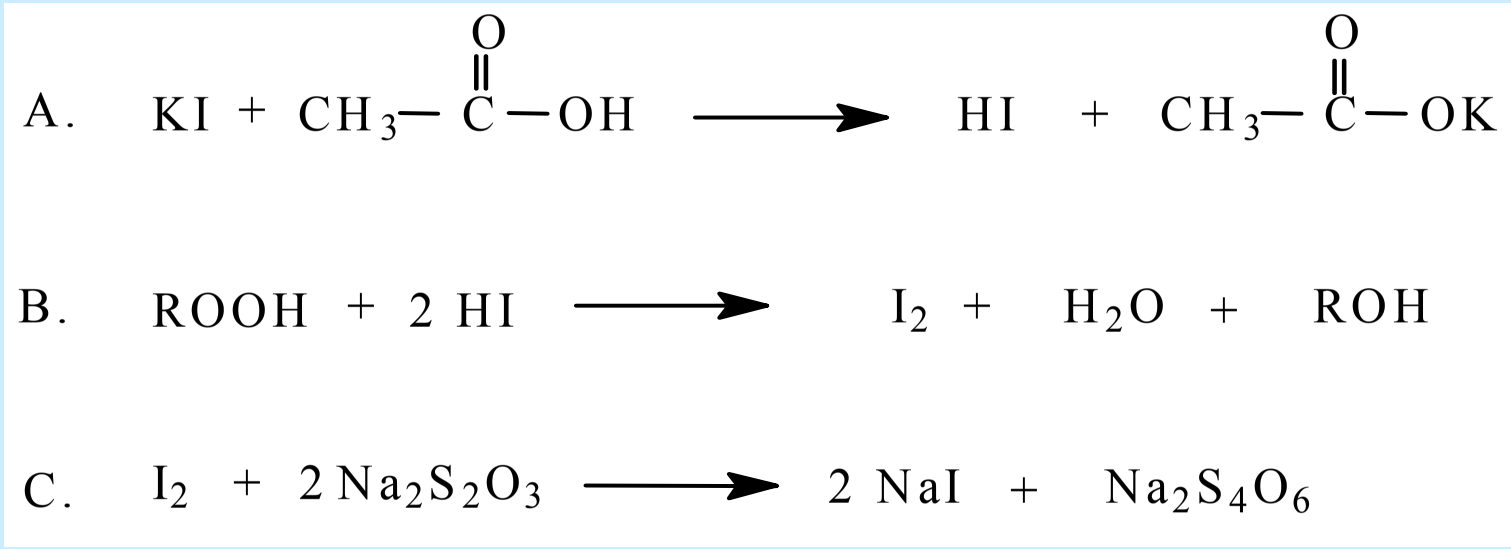
Different tests have been developed to measure the extent of autoxidation, but each respective test is like a snapshot at the time tested and do not necessarily show the complete picture.


Quantity of Reactants and Products
Secondary oxidation products
Peroxides
Primary oxidation products
Induction period*
TIME
*Induction period is the length of time before detectable rancidity, after which there is an accelerated lipid oxidation


Quantity of Reactants and Products
oygen uptake
peroxides (primary products)
time when rancid
secondary products
induction period
Time

ANALYSIS OF FLAVOR QUALITY & STABILITY OF OIL

Peroxide Value



$$\text{Peroxide Value} = \frac{\text{ml of Na2S2O3} \times \text{N} \times 1000}{\text{Grams of Oil}}$$

(milliequivalent peroxide/kg of sample)

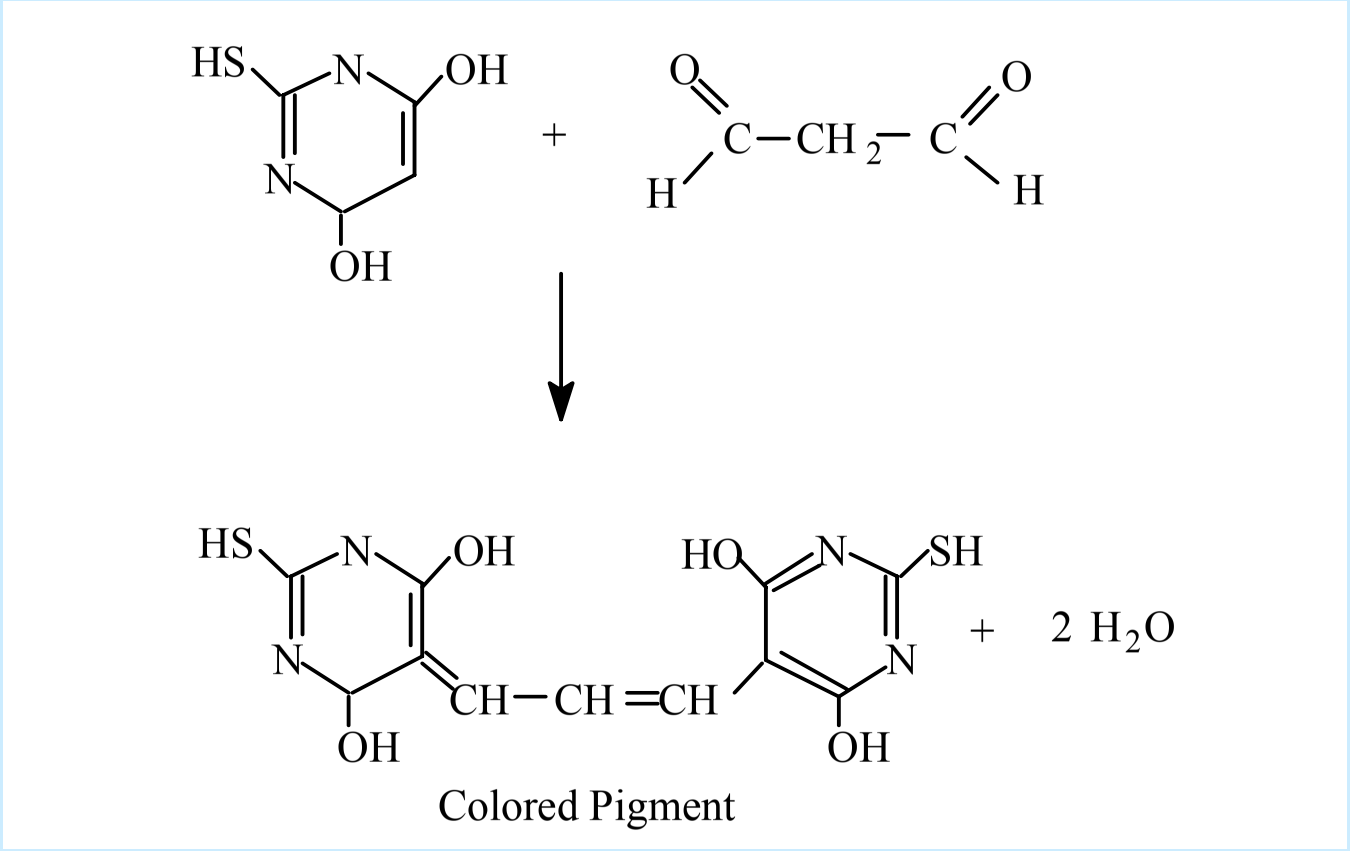
Active Oxygen Method (AOM)

Determined the time required to obtain certain peroxide value under specific experimental conditions.

The larger the AOM value, the better the flavor stability of the oil.

TBA Test

To determine the rancidity degree of meat or fish product.



Storage stability (shelf-life):

Lipids vary in their susceptibility to rancidity because of inherent properties like unsaturation, presence of antioxidants, and some external factors like processing and storage conditions.Accelerated tests are used for predicting future behaviour. For example, by raising the temperature, you naturally accelerate the rate of deterioration. At the end of the induction period, you stop and perform peroxide determination analysis.

End of shelf life can be defined as days at 20°C to reach P.V.=20. This point is generally the end of the induction period, and the end of the test. The longer the IP, the better the storage stability and the longer the expected shelflife. For this purpose many different "accelerated shelf-life" methods have been developed:

-Schaal Oven test: Oil is kept at 63°C till rancidity is detected or till the end of incubation period(Assumption:1 day of incubation at 63°C corresponds to 10 days at 20°C.)

-Active oxgyen method (AOM): O_2 is bubbled through dried and filtered oil to further accelerate autoxidation at 110°C, this is carried out till PV increases to 20 for lard and 100 for vegetable oils. The induction period is determined by discontinuous measurements of peroxide value or sensory evaluation of rancis odor.

OXIDATIVE STABILITY INDEX(OSI)

Oxidative Stability Index test, unlike tests such as peroxide value which determine oil quality only at the time of the test, has **predictive** value, thereby allowing quality decisions to be made before spoilage becomes a problem.

The 0SI is an invaluable quality control tool for eliminating inferior ingredients (for example hazelnuts for chocolate manufacture), for measuring and comparing the stability of finished oil-fat products as well as low moisture snacks like patato chips, for evaluating antioxidant activity, for monitoring the deterioration of cooking oils with use, and for many other applications where oxidative rancidity is the cause of product failure. Because the OSI has predictive value, problems are detected and eliminated before they cause rejection, unlike other tests which merely indicate that problems have occurred after it is too late to correct them.

-RANCIMAT Method: Rancimat value at Temperature T° is the number of hours,t, before the oil reaches the end of its induction period. This device performs shelf life analysis for at least 2 preselected high temperatures (i.e. 100 ° and 130 ° or 140°C).

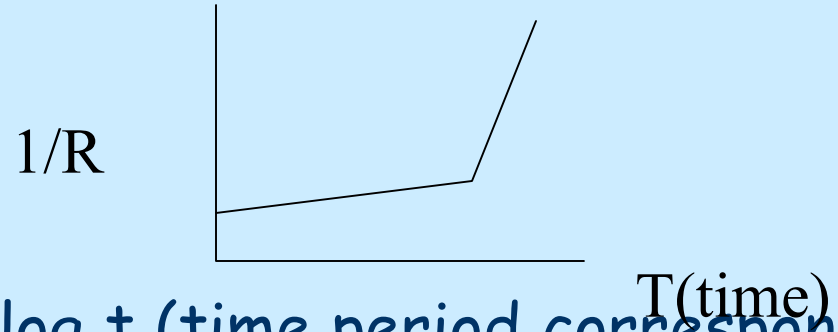
For this purpose, you put the sample in the device, increase the temperature to the preselected T_1, and the products of oil oxidation under these conditions are collected by distillation into a water trap(a special collecting vessel containing deionized water). The conductivity of water is continuously measured and recorded. The abrupt change in conductivity marks the end of the induction period,t.

Assumptions:

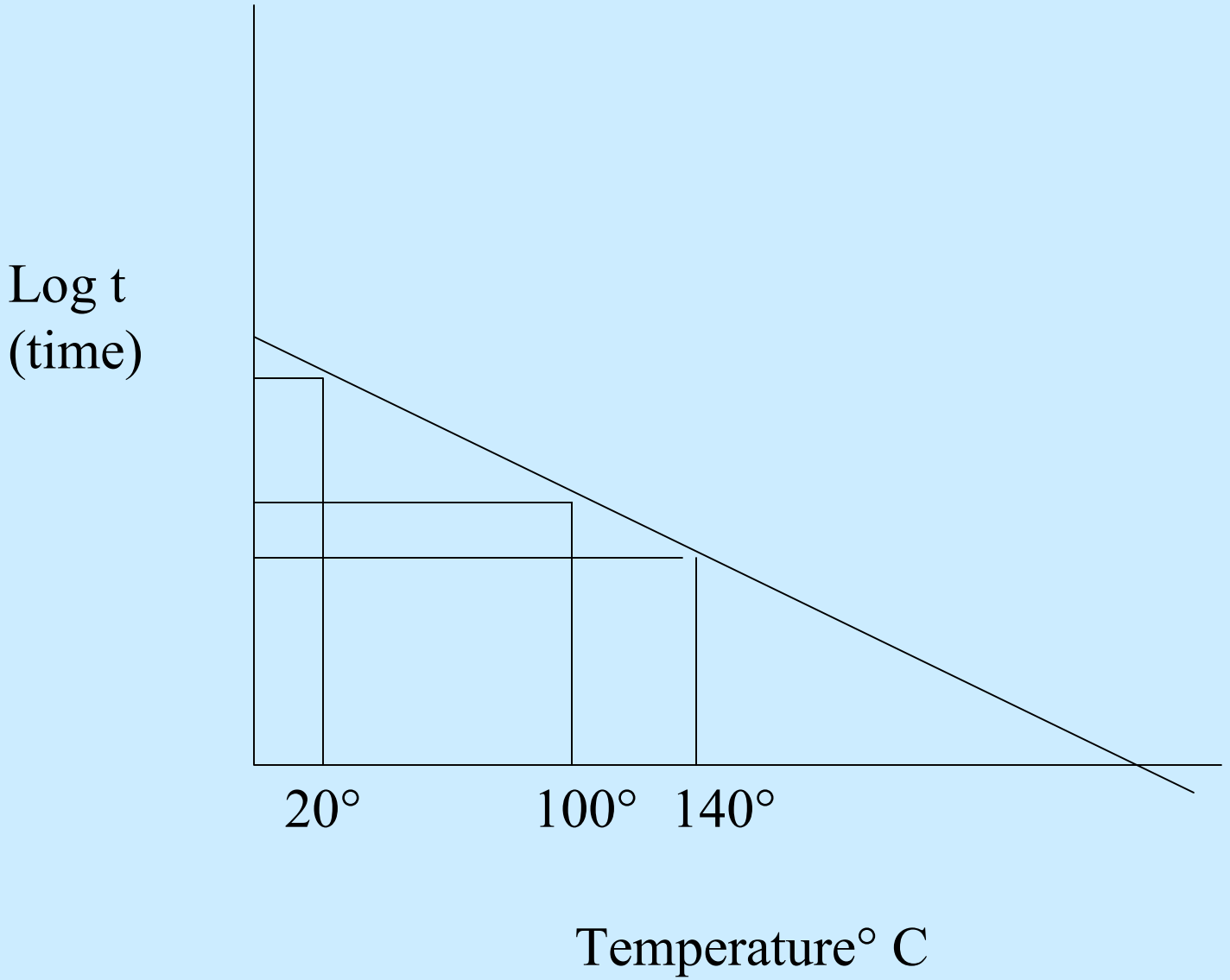
Q_{10} = rate at (T+10) °C/ rate at T °C

= shelflife at T °C / shelflife at (T+10) °C

Graphs where Conductance value(1/R) is plotted against time(t) are used for shelflife prediction.



A plot of log t (time period corresponding to end of shelf-life) versus T°C throughout a range of temperatures will be a straight line. The two points corresponding to logt1 and log t2 values at temperatures T_1 and T_2 respectively will be plotted and the two points so connected as to intercept both x-axis and y-axis. The shelf life at a third temperature T_3 can then be read from graph by extrapolation.


Log t
(time)
20°
100°
140°
Temperature° C

For refined edible vegetable oils, Rancimat values can be used in the general equation of line to deduce expected shelf-life:

$D_{20} \cong 7.40\ R_{100} - 7.9$ (here, r =0.933)

Where D_{20}=days at 20°C to reach PV=20

And R_{100} =Rancimat value in hours at 100 °C

Physical Methods-Thermal Methods

*Differential scanning calorimeter

*Thermogravimetric and pressure differential calorimetric methods

*Oxidative stability-length of the incubation period-time to max weight gain and rate of weight increase-time of max enthalpy change

Thermal Method-
Chemiluminescence
*Determine the content of effectiveness of antioxidants in oils and fats
*correlates with the peroxide value and anisidine values and flavour
*emitted during peroxidate breakdown

Indicators of Quality of Heated Oils

*Deteriotive changes-thermal oxidation,hydrolysis, polimerization

*Deep fat frying potatoes with fast turnover in sunflower oil-thermoxidative changes

*A dramatic rise in polar compounds; More than 25-27% polar lipids should be rejected

*NIRS-determine the sum of dimer and polymer triglycerides and acid value to evaluate frying oil

Changes in dielectric constant

Toxic Contaminants and Adulterants

-Contamination by weed seeds during harvesting

-Storage of safe oil in unsafe container

Contamination due to Faulty Storage

*Contamination of vegetable oils by mineral oils- determining the saponification values of the lubricating oil samples

*

Indices of Admixtures, Blends, Contaminants and Adulterants-One Fat to Another

*Stable carbon isotope ratio analysis-establishing the authenticity of oils

*Fourier transform infrared spectroscopy for authenticity of extra virgin olive oil

Admixture of Vegetable Oils with Other Vegetable Oils

Biosynthesis of Fat

-Species specific

-Detection of adulteration can be based on the basis of various constituents in the fat admixture

Admixture of Vegetable Oils with Other Vegetable Oils

-Fatty Acid Composition

-Triglyceride Analysis

-Unsaponifiable Fraction of Oil

*Sterol analysis

*Tocopherol analysis

*Phenolics and Alcohols

Fatty Acid Composition

*Without being detected by the routine physical and chemical characteristics-GLC analysis of fatty acids prove useful-presence of soybean oils in olive oil

*IOOC for olive oil purity-saturated fatty acid content at position-2

*Pancreatic lipase allows the differentation between virgin and esterified olive oils-Saturated fatty acid level <1,5% in virgin olive oil

Fatty Acid Composition

- *Coconut oil -determination of saponification and IV-detect gross and simple adulteration
- *Analysis of methyl esters of fatty acids by GC-ratios of certain fatty acids-detection of 5% rapeseed oil in peanut oil(C22-C16)
- Palm kernel and coconut oils in adulterated cocoa butteras well as addition of coconut or palm kernel oil to butter (high lauric and myristic acid content).
- *Linolenic acid-detection of 5% grapeseed oil or <5% sunflower oil in olive oil

Unsaponifiable Fraction of Oils

*The presence or absence of certain individual unsaponifiable components can help in establishing the identity of each oil (authenticity or genuineness) and also in detection of admixture with another oil.

Unsaponifiable Fraction of Oils Sterol Analysis

*Analysis of sterols from unsaponifiable fraction -adulteration olive oil with soybean oil

*Sterol composition obtained by fraction of the unsaponifiable matter is characteristic of virgin olive oils

*The ratio of the content of β-sitosterol to the sum of (stigmasterol and campesterol) is 25-30 for virgin olive oil.

Virgin olive oil has β-sitosterol content of 94% or higher

Unsaponifiable Fraction of Oil Tocopherol Analysis

*Concentration of γ-tocopherols in solvent extracted cottonseed oils increased on contamination with soybean oil

*Contamination of olive oil with peanut oil -detected from ingreased β-tocopherol levels

*Quantitative determination of δ and γ-tocopherols -a method for determining the adulteration of olive oil with less expensive oils

Unsaponifiable Fraction of Oil Phenolics and Alcohols

*Reverse phase HPLC with UV detection and GC-MS-quantification of phenols

*Olive oil press cake in olive oil can be detected on the basis of the presence of higher fatty alcohols in the former

Blends of Vegetable and Marine /Animal Fats Fatty Acid Composition

*GLC analysis of fatty acids composition-detect admixture of marine oils in vegetable oils (increased myristic, palmitoleic, C20 and C22 acid contents).

*Purity and contamination of vegetable oils with lard-values of methyl esters of C8-C20 fatty acids

Fatty Acid Composition

*Animal fats contain tetradecanoic and hexadecanoic acids in greater amounts than does olive oil

*Average molecular weight of the distilled fraction from pure olive oil has a saponification number less than 191-increases with adding animal fats

Unsaponifiable Fraction

*Differences in the sterol composition of animal and plant fats-an efficient method for detecting admixture

*Adulterant animal fat at % 10 in vegetable oil- detected on basis of cholesterol

Detection of Mixtures of Animal Fats

*Detection for adulteration of beef fat by pig lard-fatty acid composition and physical characteristics

*Detection fo interspecies meat adulterationis very often on the differences in fat characteristics

Sensory Quality of Oils

"Global" quality evaluation of virgin olive oil the IOOCproposed (1989) th following equation:

O.Q.I = 2,55 + 0,91SE - 0,78AV

7,35K270 -0,066PV

O.QI: Over-allquality index

SE:Sensory evaluation (from3,9-9,0)

AV:Acid value (from 0,1-3,3)

K270 :Absorbance at 270 nm

PV:Peroxide value(from 1,0-20,0)