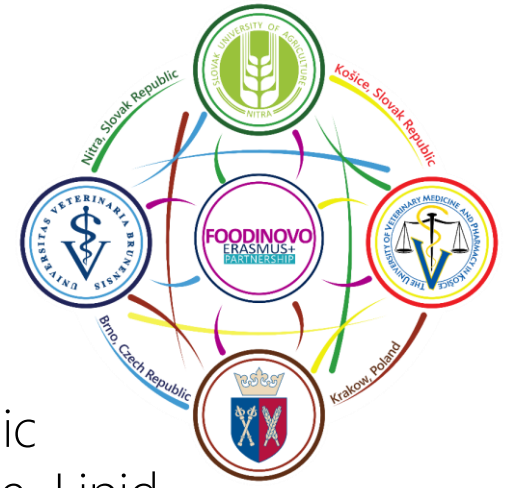
Fat and oil identification

Co-funded by the
Erasmus+ Programme
of the European Union

Definition of Lipids



- There is no "standard" definition of lipids, although they often mention solubility
- "a wide variety of natural products including fatty acids and their derivatives, steroids, terpenes, carotenoids and bile acids, which have in common a ready solubility in organic solvents such as diethyl ether, hexane, benzene, chloroform or methanol." W. W. Christie. Lipid Analysis. Pergamon Press, New York, 1982, p. 1.
- "those substances which are (a) insoluble in water; (b) soluble in organic solvents such as chloroform, ether or benzene; (c) contain long-chain hydrocarbon groups in their molecules; and (d) are present in or derived from living organisms." M. Kates. Techniques of Lipidology: Isolation, Analysis and Identification of Lipids. Elsevier, New York, 1986, p. 1.
- "a chemically heterogeneous group of substances, having in common the property of insolubility in water, but solubility in non-polar solvents such as chloroform, hydrocarbons or alcohols." M. I. Gurr and A. T. James. Lipid Biochemistry and Introduction. Cornell University Press, Ithaca, NY, 1971, p. 1.
  - C1–C4 very short chain fatty acids (VSCFAs) are soluble in water and insoluble in nonpolar solvents
  - some trans fatty acids are not derived directly from living organisms.

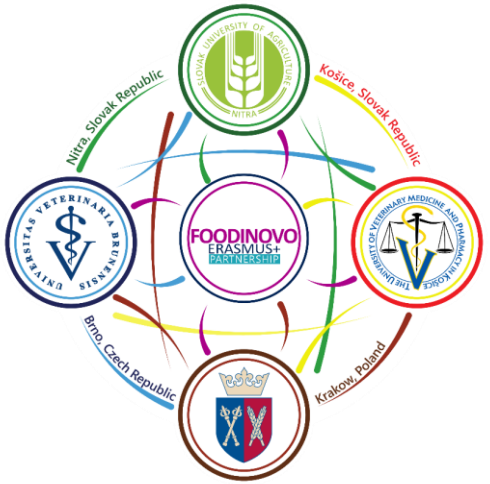
Lipid classification

- Physical form under ambient conditions:
  - Oils (liquid)
  - Fats (solid)
- Structure
  - Simple (acylglycerols, ether acylglycerols, sterols, and their esters and wax esters)
  - Complex/Composite (glycerophospholipids (phospholipids), glyceroglycolipids (glycolipids), and sphingolipids)
  - Derived (building blocks of the above-mentioned groups)
- Polarity
  - Polar
  - Neutral
- Nutritional requirements
  - Essential
  - Non-essential

Lipid classes

- Fatty acids
  - Saturated, unsaturated (including trans, cis, acetylenic), branched, cyclic, hydroxy and epoxy, furanoid
- Acylglycerols
- Sterols and sterol esters
- Waxes
- Phosphoglycerides (Phospholipids)
- Ether(phospho)glycerides (Plasmalogens)
- Glyceroglycolipids (Glycosylglycolipids)
- Sphingolipids
- Fat-Soluble Vitamins
  - A, D, E, K
- Hydrocarbons

Nomenclature systems of fatty acids



- Standard (IUPAC)
  - fatty acid is named after the parent hydrocarbon
  - Double bonds are designated using the Δ configuration, which represents the distance from the carboxyl carbon, naming the carboxyl carbon number 1 (Δ is often omitted).
  - Example - cis-9-octadecenoic acid
- Common (Trivial)
  - oleic acid
- Shorthand (ω)
  - 18 : 1ω9

Food Applications of Lipids

- Application aspects of lipids:
  - Culinary - heat transfer media, flavor carriers, texture/mouthfeel agents
  - Nutritional - provide energy (1 g: 9 kcal, 30% of total calories in a diet), essential fatty acids, oil-soluble vitamins and other physiologically important compounds
- Isolation & purification of fats and oils
  - Recovery – extraction/crushing (plants), rendering (animal fats)
  - Refining – physical/chemical methods
  - Conversion – hydrogenation, winterizing, fractional crystallization, and interesterification
  - Stabilization - plasticizing, tempering, and stehling

Lipids in foods

- Fat contents of selected foods:
  - Liquid vegetable oils = 100%,
  - Margarine and butter = 80% (emulsions of lipid in water),
  - Almonds: 55%,
  - Walnuts: 65%,
  - Cereals: 3-5%,
  - Sunflower seeds, hulled : ~60%,
  - Chocolate: ~35%,
  - Milk: 3.7%,
  - Sausages: 20-37%,
  - Milk powder: 27.5%,
  - Tuna: 4% (some fish contain 15% fat)

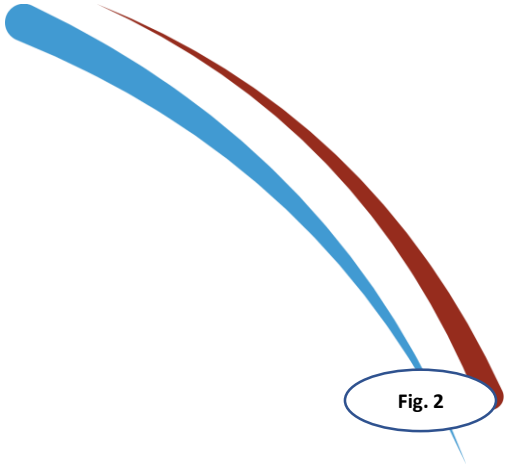


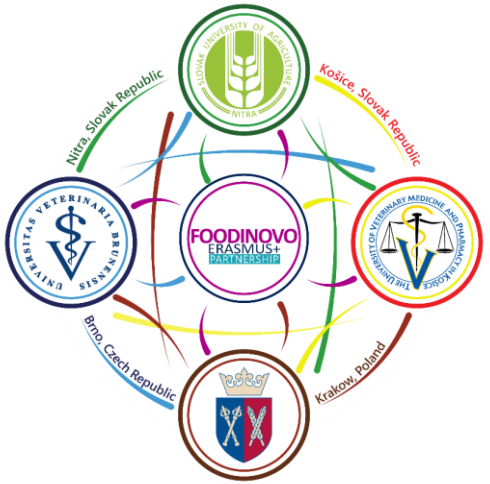
Fig. 2

Physical properties of oils and fats

- The organoleptic evaluation of fats and oils is difficult and subjective.
- Physical properties allow to formulate rapid tests, which should correlate with internationally recognized standard methods, provide an objective index, be easy to use, quantify the degree of oil degradation and be safe for use in food processing.

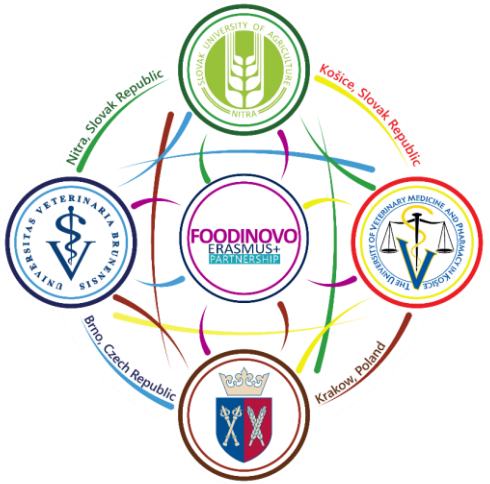
- Density (around 0.9 g/cm^3)
- Phase transitions temperatures (DSC), cloud point, melting temperature
- Viscosity, rheology, texture
- Crystalline structure of fats (X-ray)
- Refractive index (around 1.41)
- Solid fat index (by change in specific volume with respect to temperature - dilatometry)
- Solid fat content (by pulsed nuclear magnetic resonance)
- Dielectric constant,
- Smoke/burning point,
- Dropping point (grease)
- Color,
- Foaming properties
- UV-absorption

Bulk Oil/Fat Properties



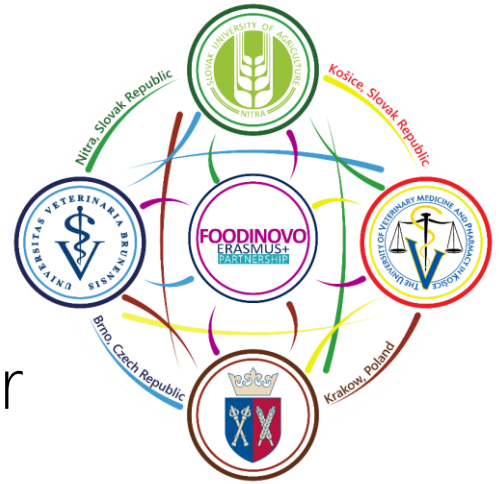
- Degree of unsaturation
- Free fatty acid content
- Saponification value
- Volatile compounds
- Oxidative stability
  - Peroxide number
  - Thiobarbituric acid (TBA) value
  - Anisidine value
  - Totox value

Degree of Unsaturation



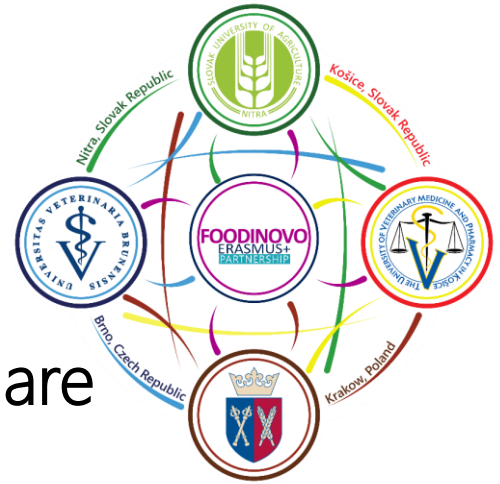
- Iodine value - the number of grams of iodine absorbed by 100 g of lipid
  - reasonable if the double bonds are not conjugated with each other or with a carbonyl oxygen
  - should be carried out in the absence of light
- IR spectrum - 1500–900 cm^{-1} - a band due to the CH=CH of isolated trans double bonds

Free fatty acid content



- Indication of insufficient processing, lipase activity, or other hydrolytic actions (for frying oils should be below 0.05%)
- Acid value - the number of milligrams of KOH required to neutralize the free acids in 1 g of sample (0.6 mg/KOH/g oil for refined oils; 4 mg/KOH/g cold pressed or virgin oils), questionable for detection of oil deterioration during frying
- Alternatives:
  - colorimetrical determination of copper complexes (640 - 690 nm) after dissolving oil in chloroform
  - FTIR (COOH group in the center region of mid-IR)

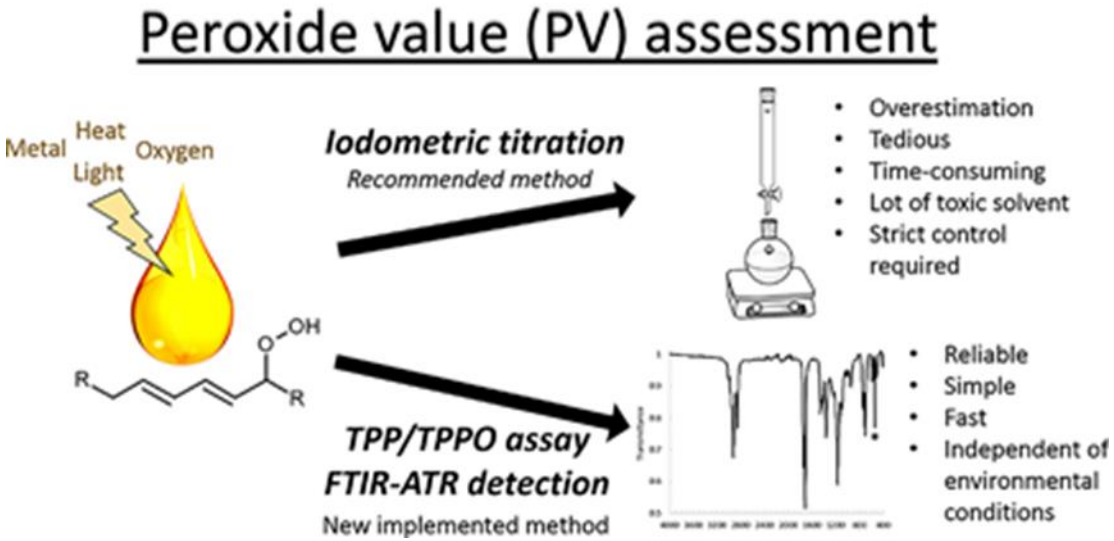
Saponification number



- Milligrams of KOH required to saponify 1 g of fat (triglycerides are hydrolyzed under excess of alkali; back titration with HCl)
- High values indicate short fatty acids, and small molecular mass of triglycerides $\mathrm{MW}_{\mathrm{oil/fat}} = 3 \times 56,106 \div \mathrm{SV}$
- Ester value - the difference between the saponification number and the acid value (indicates real degree of saponification in contrast to neutralization of free fatty acids)

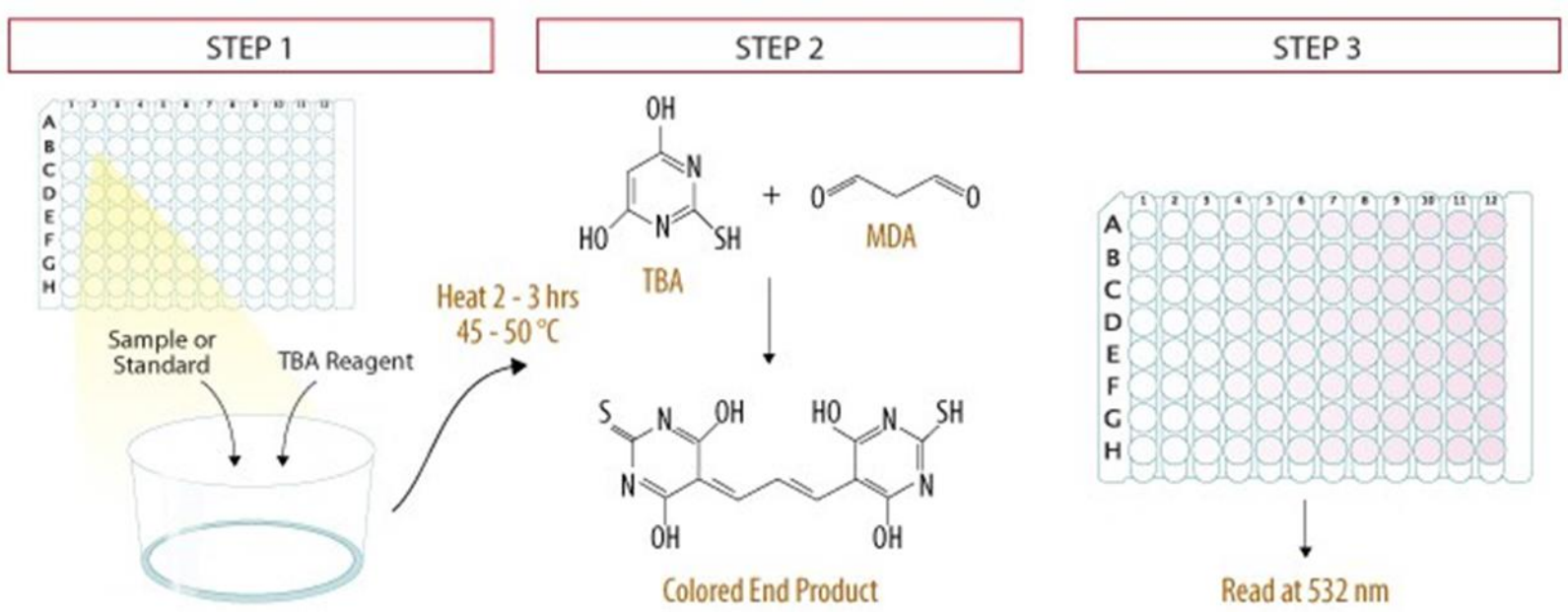
Peroxide value (PV/POV)

- Hydroperoxides (LOOH)
  - first stable products of lipid oxidation by free radicals
  - the most commonly quantified products
- PV – the amount of peroxide (in milliequivalents, mEq) per 1 kg of sample (Codex Alimentarius allows10 meq/kg oil for refined oils and up to 20 meq/kg of cold pressed or virgin oil)



https://doi.org/10.1002/ejlt.201800109

2-Thiobarbituric Acid Value

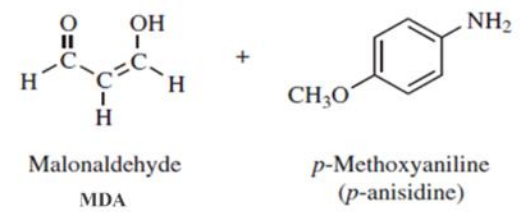


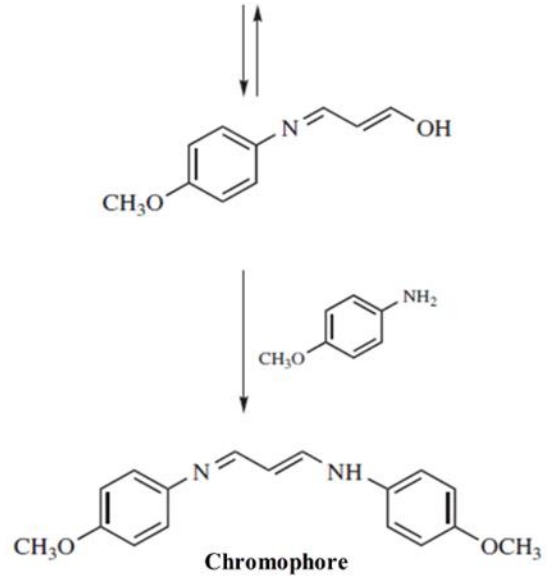
https://www.rndsystems.com/cn/product-highlights/tbars-parameter-kit-measuring-oxidative-stress

TBA value - milligrams of malonaldehyde (MA) equivalents per kilogram sample or as micromoles MA equivalents per gram sample
TBA-reactive substances (TBARSs)

p-anisidine value (AV)

- Reaction with aldehydes (principally 2-alkenals and 2,4-alkadienals)
- AV (pAnV): 100 times the optical density measured at 350 nm in a 1.0-cm cell of a solution containing 1.0 g of oil in 100 mL of a mixture of solvent and reagent
- Correlates with sensory scores





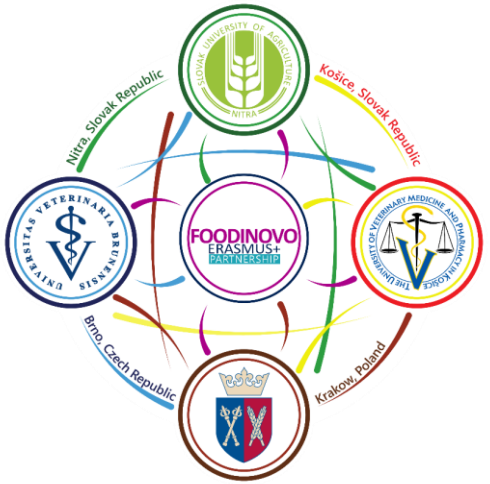
TOTOX Value

- Overall index of primary (LOOH) and secondary (aldehydes) oxidation products
- TOTOX value = 2PV + p-AnV (warning: different dimensions)
- In cases, where no AV could be measured TBA value could be used instead (similarly - total carbonyl content measured by a colorimetric method with 2,4-dinitrophenylhydrazine; DNPH).

Sources of oils and fats

- Total production of edible lipids – 230 mln tons (2020)
- Vegetable oils/fats - 88% of world supply
- Animal fats - 11% (lard, tallow, butter/ghee)
- Marine & microbial sources – below 1%
  - whale
  - fish (shark, cod, tuna, sardines)
  - seaweeds
  - krill, microalgae

Plant Oils and Fats

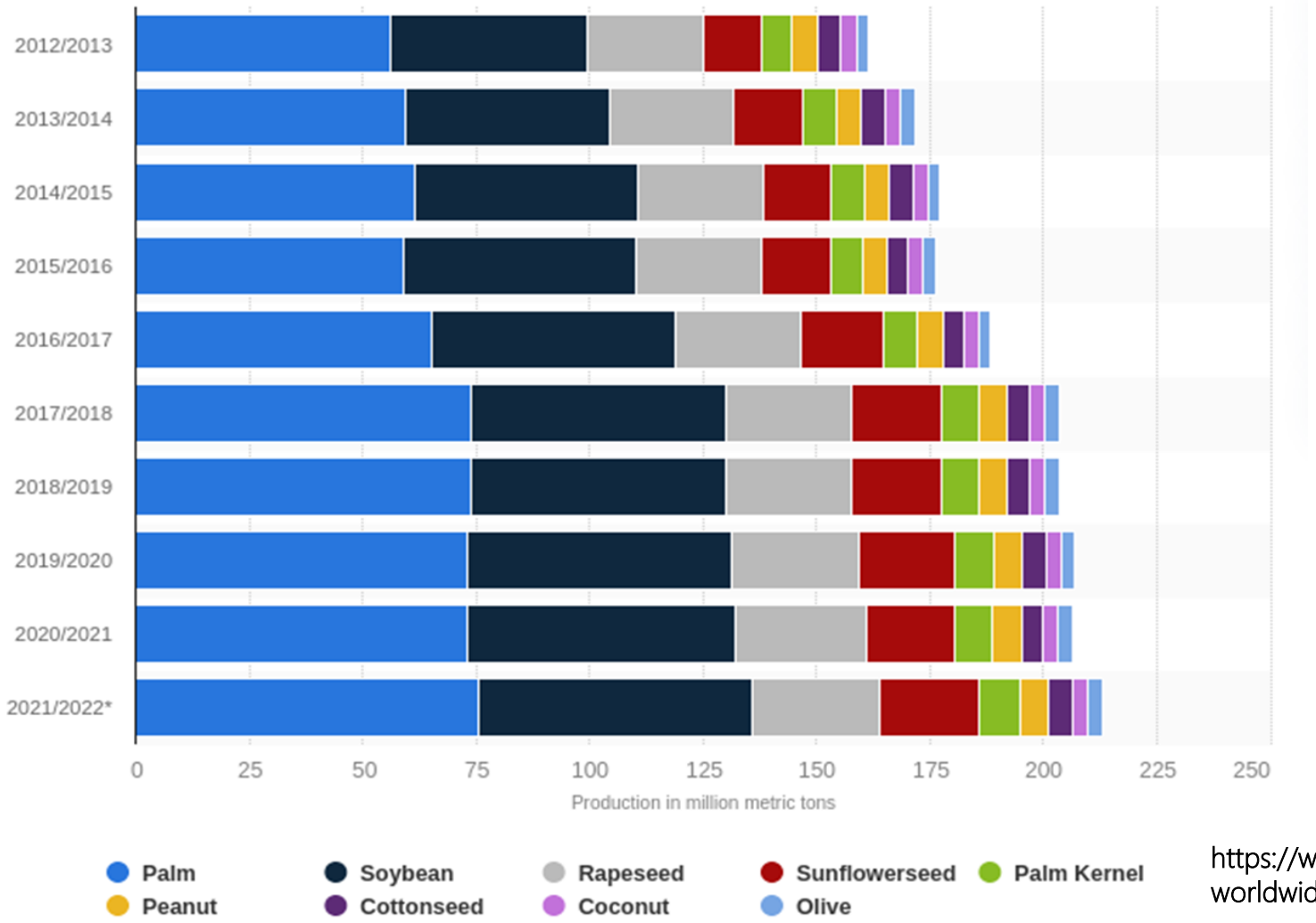


- Pulp oils: palm oil, olive oil, avocado oil
- Lauric oils: coconut oil, palm kernel oil, babassu oil, laurel oil, nutmeg oil, dika butter
- Fats rich in palmitic and stearic acid: cocoa butter, illipe butter, mowrah butter, shea butter, borneo tallow
- Seed oils rich in palmitic acid: cottonseed oil, cereal germ oils, corn oil, pumpkin oil
- Seed oils rich in oleic and linoleic acid: sesame oil, sunflower oil, safflower oil, niger oil, linseed oil, poppy-seed oil, grape-seed oil, walnut oil, fruit-seed oils, berry-seed oils, tea-seed oil
- Oils of *Leguminosae*: peanut oil, soybean oil, lupine oil
- Oils of *Cruciferae*: rapeseed oil, mustard-seed oil

Recovery of oil from plant materials

- Storage of oilseeds
- Cleaning
- Dehulling
- Conditioning
- Flaking
- Pressing/Extraction
  - Hard screw pressing
  - Prepress solvent extraction
  - Direct solvent extraction

Production of major vegetable oils



https://www.statista.com/statistics/263933/production-of-vegetable-oils-worldwide-since-2000/

Herbaceous oil-bearing plants

No.	Common name	Species	Genus	Familia	Main producing area	Oil content	References
1	Soybean	*Glycine max (Linn.) Merr.*	*Glycine*	*leguminosae* sp.	China, the United States, Brazil et al.	18–24%	(Li et al., 2018)
2	Rape	*Brassica napus* L.	*Brassica*	*Brassicaceae*	All over the world	37.5–46.3%	(Zhao et al., 2005; Ishaq et al., 2017)
3	Sunflower	*Helianthus annuus*	*Helianthus*	*Compositae*	All over the world	46–50%	(Rauf et al., 2017)
4	Peanut	*Arachis hypogaea* L.	*Arachis*	*leguminosae* sp.	Asia, Africa, America, et al.	46–57%	(Wang X. et al., 2018)
5	Cotton	*Gossypium spp*	*Gossypium*	*Malvaceae*	China, the United States, India, Uzbekistan, Egypt, etc.	15–40%	(Shang et al., 2017)
6	Corn	*Zea mays* L.	*Zea*	*Gramineae*	Tropical and temperate regions of the world	4.5–4.8%	(Wang et al., 2010)
7	Sesame	*Sesamum indicum*	*Sesamum*	*Pedaliaceae*	Tropical and temperate regions	43–61%	(Latif and Anwar, 2011)
8	Hemp	*Cannabis sativa L. subsp. sativa*	*Cannabis*	*Moraceae*	All over the world	25–35%	(Vonapartis et al., 2014)
9	Grape	*Vitis vinifera* L.	*Vitis*	*Vitaceae*	All over the world	10–20%	(Movahed and Ghavami, 2007)
10	Fiberflax	*Linum usitatissimum* L.	*Linum*	*Linaceae*	Mediterranean region, Euro-Asian Temperature Zone	35–45%	(Martinchik et al., 2012)
11	Safflower carthamus	*Carthamus tinctorius* L.	*Chelonopsis*	*Labiatae*	China, Russia, Japan, North Korea, et al.	About 40%	(Toma et al., 2014)
12	Rice	*O. sativa*	*Oryza*	*Poaceae*	Almost everywhere, expect Antarctica.	15–23%	(Ju and Vali, 2005)
13	Perilla	*Perilla frutescens (L.) Britt.*	*Perilla*	*Labiatae*	India, Myanmar, Japan, Korea, Indonesia, Russia, et al.	40–50%	(Liao et al., 2018)

https://www.frontiersin.org/articles/10.3389/fpls.2020.01315/full

Woody oil-bearing plants

No.	Common name	Species	Genus	Familia	Main producing area	Oil content	References
1	Oil palm	*Elaeis guineensis* Jacq.	*Elaeis*	*Arecaceae*	Tropical regions of Africa, tropical regions of China, Taiwan, Hainan and Yunnan.	50–55%	(Kasemsumran et al., 2012)
2	Coconut	*Cocos nucifera* L.	*Cocos*	*Arecaceae*	Asia, Africa and Latin America	65–74%	(Marina et al., 2009)
3	Olive	*Olea europaea* L.	*Olea*	*Oleaceae*	Mediterranean coast	31–56%	(Sun et al., 2017; Olmo-García et al., 2018)
4	Tea-oil tree	*Camellia oleifera Abel*	*Camellia*	*Theaceae*	From Yangtze River Valley to Southern China	47.0–59.5%	(Chen et al., 2011)
5	Walnut	*Juglans regia* L.	*Juglans L.*	*Juglandaceae*	Southeastern Europe, Himalaya mountains, China	50–70%	(Özcan et al., 2010)
6	Peony	*Paeonia suffruticosa* Andr	*Paeonia*	*Paeoniaceae*	Henan, Sichuan, Tibet, Guizhou, Yunnan of China	27–33%	(Ning et al., 2015; Zhang et al., 2018)
7	Pecan	*Carya cathayensis* Sarg.	*Carya*	*Juglandaceae*	Anhui and Zhejiang, China	60–70%	(Huang et al., 2016)
8	Hazelnut	*Corylus heterophylla* Fisch.	*Corylus*	*Betulaceae*	Temperate zone in Asia, Europe and North America	50–75%	(Balta et al., 2006; Miraliakbari and Shahidi, 2008; Juhaimi et al., 2018)
9	Idesia	*Idesia polycarpa* Maxim.	*Idesia*	*Flacourtiaceae*	Southwest China, North Korea, South Japan.	21.2–44.0%	(Zhu, 2010; Gong et al., 2012; Li R. J. et al., 2016)
10	Pine	*Pinus*	*Pinus*	*Pinaceae*	Brazil, coniferous forests, et al.	58–69%	(Ryan et al., 2006; Bao and Guo, 2016)
11	Cocoa	*Theobroma cacao* L.	*Theobroma*	*Sterculiaceae*	Narrower within 10°north-south latitude of the equator	45–60%	(Servent et al., 2018)
12	Shiny-leaved yellowhorn	*Xanthoceras sorbifolium Bunge*	*Xanthoceras Bunge*	*Sapindaceae*	North and northeast China	50–60%	(Cao, 2015)
13	Acer truncatum	*Acer truncatum* Bunge	*Acer Linn.*	*Aceraceae*	Northeast and north China, Shaanxi, Sichuan, et al.	42–46%	(Zhang and Hou, 2010)

https://www.frontiersin.org/articles/10.3389/fpls.2020.01315/full

Palm

Plant (*Elaeis guineensis* Jacq.)

- grows in wet tropical regions
- most productive source of oil (3.5t/ha)
- Oil content 50-55%

Oil

- Two different oils – major from outer part of the fruit, minor from kernels
- 10% PUFA, 40% MUFA, 50% SFA – fractionated into olein and stearin
- high oxidative stability

Soybean

Plant (*Glycine max*)

- Dicotyledon (beans splitting into halves) during conveying and transporting
- Popular target for GM
- Contains lipoxygenase (special for legumes) and chlorophyl (singlet oxidation under light causes "beany" or "grassy" flavor, due to formation of 2-pentylfuran and 2-pentenylfuran from linolenic acid)

Oil

- Cheap, worldwide available
- Residual meal contains high quality protein

https://www.seedoilpress.com/oil-production-plant/soybean-oil-production-line.html

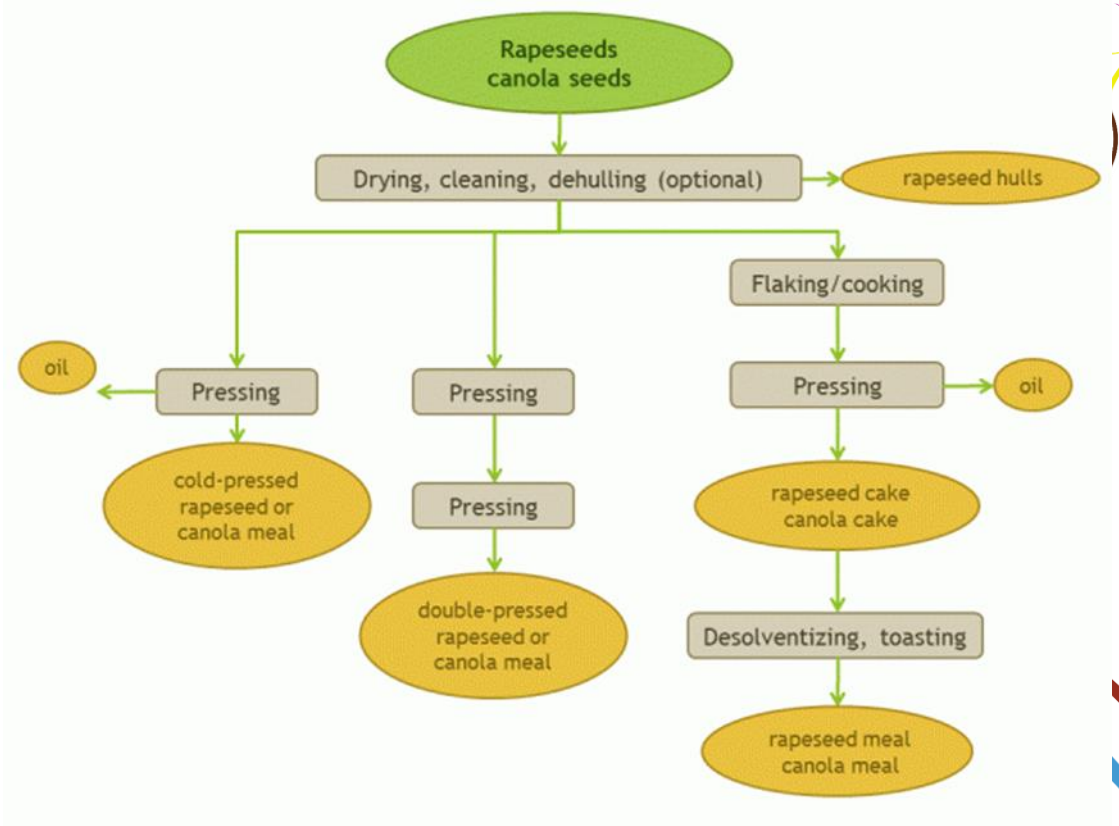
Rapeseed

Plant

Brassica species (*B. napus*, *B. rapa*, *B. juncea*)
GM prone – e.g. HORO
High-erucic cultivars still in use (for technical purposes)

Oil

low level of saturated acids,
high level of oleic acid,
presence of linoleic and linolenic acids at a favorable ratio (~ 2:1)



https://www.feedipedia.org/content/rapeseed-and-canola-seed-processes-and-products

Sunflower

Plant

Helianthus annuus

Origin – North America

Oil

three ranges of fatty acid composition

traditional (linoleic-rich)

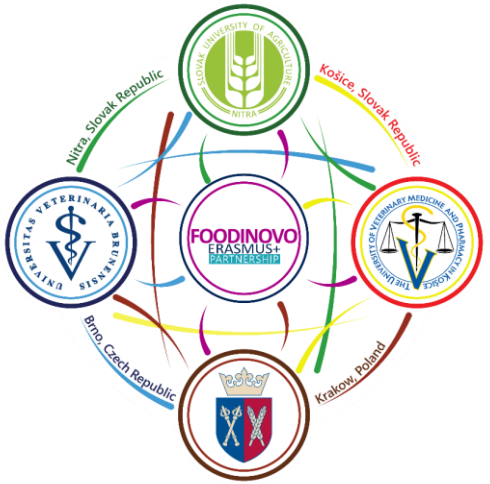
high oleic oil

mid-oleic oil

Olive oil

- Plant
  - *Olea europaea* L.
  - Olive trees bear fruit for over 100 years.
- Oil
  - Produced since ca. 5000 BC
  - Three extraction systems in use – pressure, percolation and **centrifugation**
- Extra virgin, virgin, ordinary and lampante virgin olive oils; pommace oil
- Richest traditional source of MUFA (up to 80%)– health benefits

Lipids from land animals



- Animal derived lipids:
- Animal fats (the lipids in body tissues)
- Milk lipids
- Eggs (marginal fat intake)
- Physiologically functional fatty acids:
- arachidonic (ARA)
- docosahexaenoic acid (DHA)
- The human hunger for meat, can actually be interpreted as hunger for meat fat (Harris,1985)
- It may be assumed that producing fat animals to gain animal fat was originally a major goal of planned livestock production.

Animal fats

- Tallow (ruminant species: cows, sheep, goat)
- Premier jus (oleo stock; bovine animals - heart, caul, kidney and mesentery)
- Lard (pigs)
- Pure rendered lard
- Lard subject to processing
- Rendered pork fat (bones, detached skin, head skin, ears, tails and other issues fit for human consumption)
- Choice white grease – A specific grade of mostly pork fat defined by hardness, color, fatty acid content, moisture, insolubles, unsaponifiables, and free fatty acids.
- Yellow grease – usually made up of restaurant greases (fats and oils from cooking). Another source could be from rendering plants producing lower-quality tallow, fats, and greases.
- Poultry fat

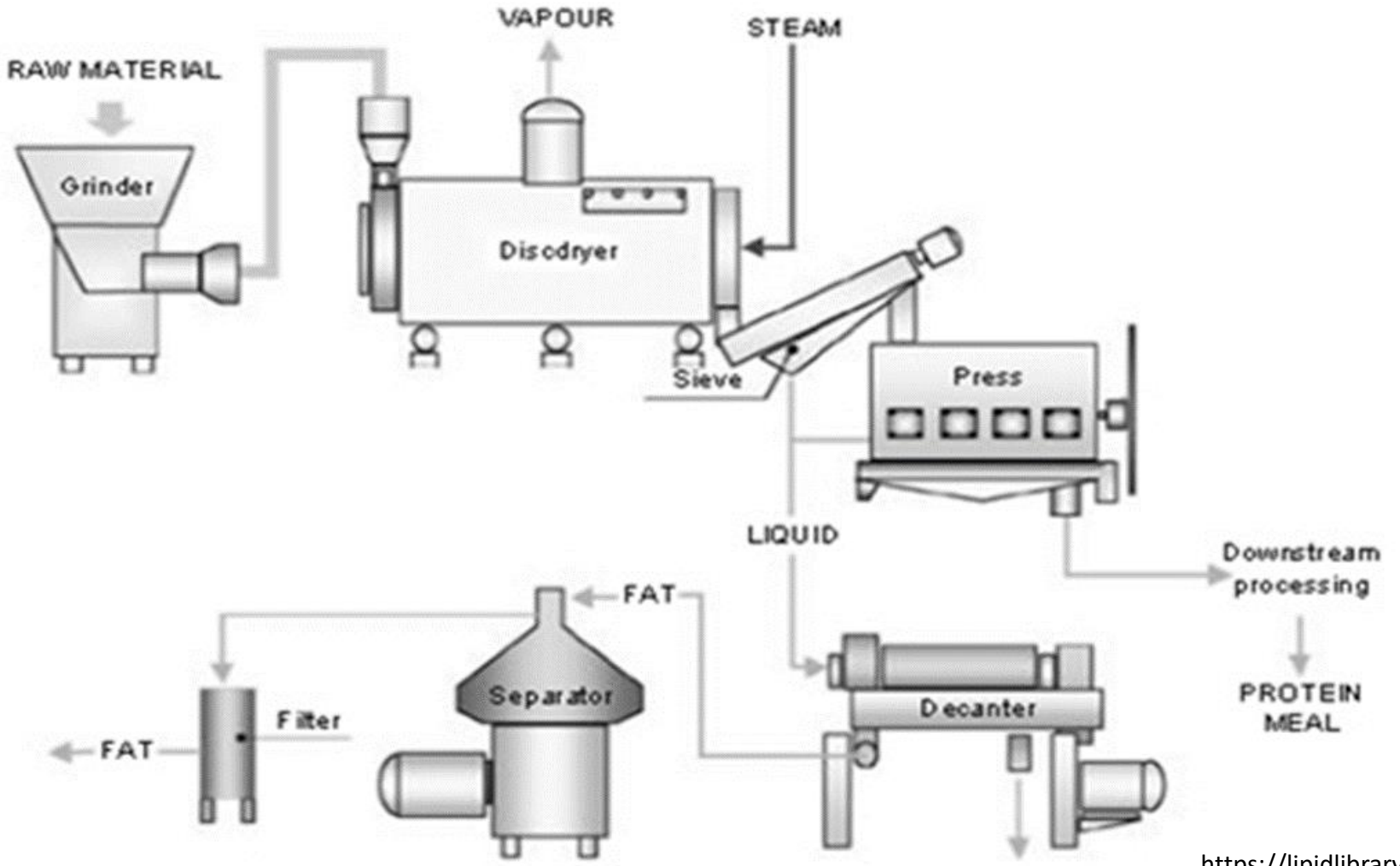
Main sources of meat fat:

- internal fat (mainly around kidneys and stomach)
- subcutaneous adipose tissue (under the skin)
- intermuscular fat (between muscles and bones)
- intramuscular fat (between skeletal muscles)

Rendering techniques:

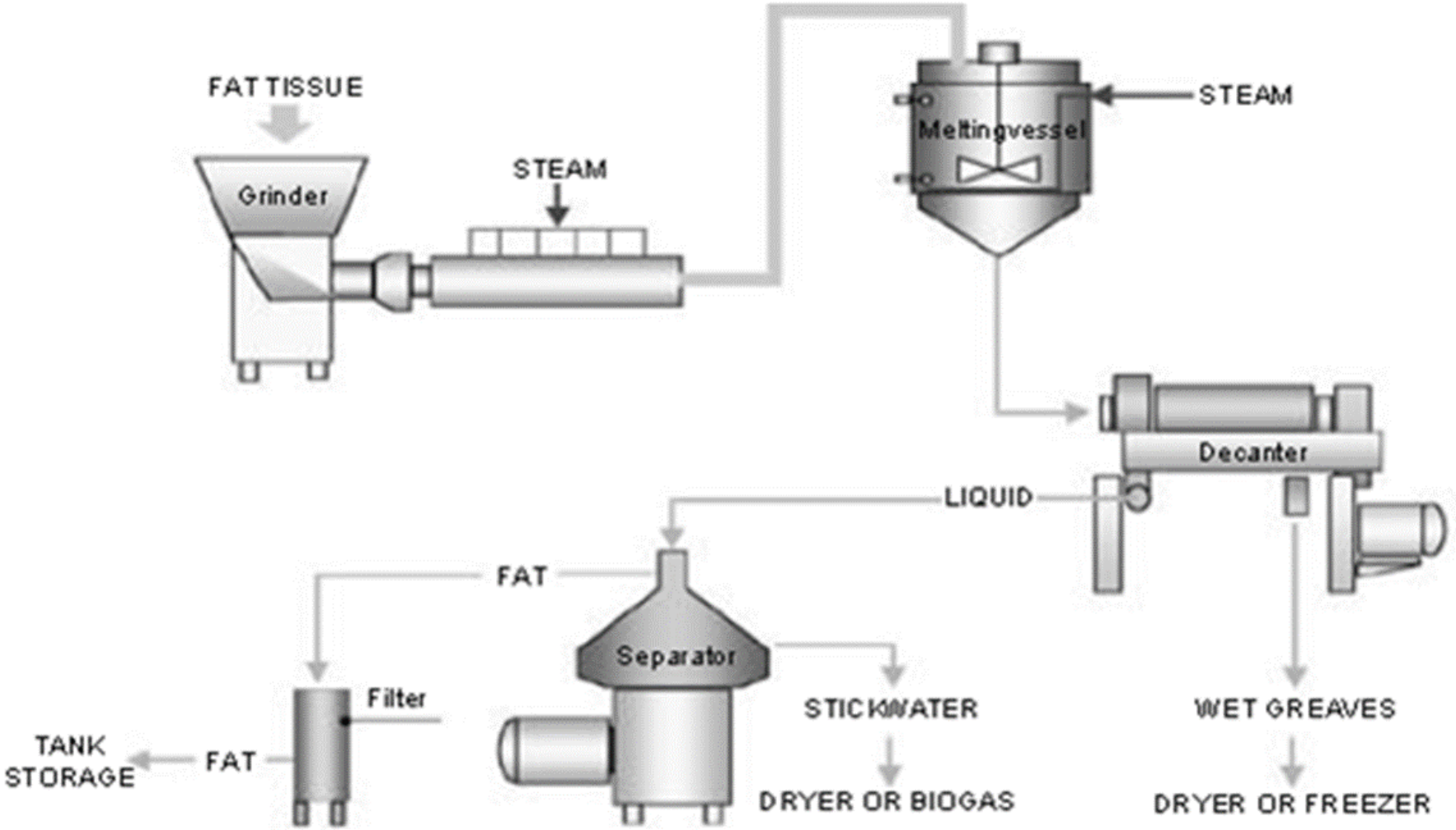
dry (simplest), wet (steam), slurry (grounding, drying; centrifugation; pressing), digestive (enzymes or chemicals

Dry rendering



https://lipidlibrary.aocs.org/edible-oil-processing/animal-fats

Wet rendering



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Selected Properties of lipids from land animals

	Iodine value	Saponification number	Melting point [°C]
Butter	25–42	210–233	28–35
Lard	53–77	190–202	33–46
Neat's-foot oil	69–76	190–199	–4
Beef tallow	40–48	190–199	40–48
Mutton tallow	35–46	192–197	44–51
Poultry fat	65-88	195-205	23-40

DUGAN L R JR (1987), Meat animal by-products and their utilization, Part 1. Meat fats, in Price J F and Schweigert B S, The Science of Meat and Meat Products, 3rd edn, Westport, CT, Food & Nutrition Press, Inc., 507–530

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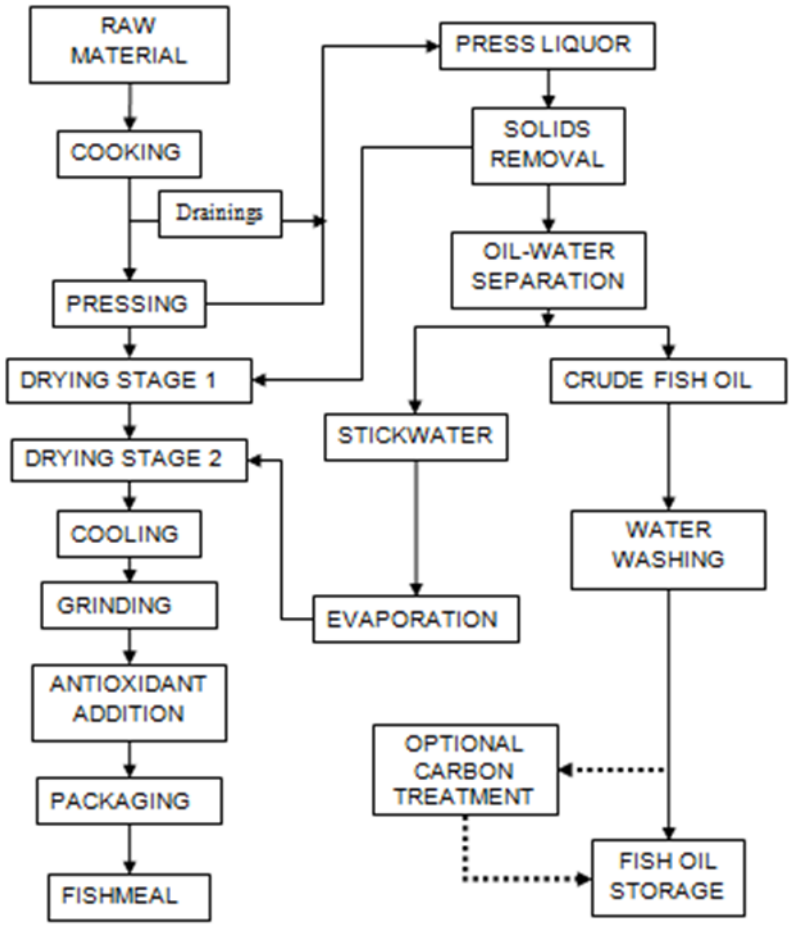
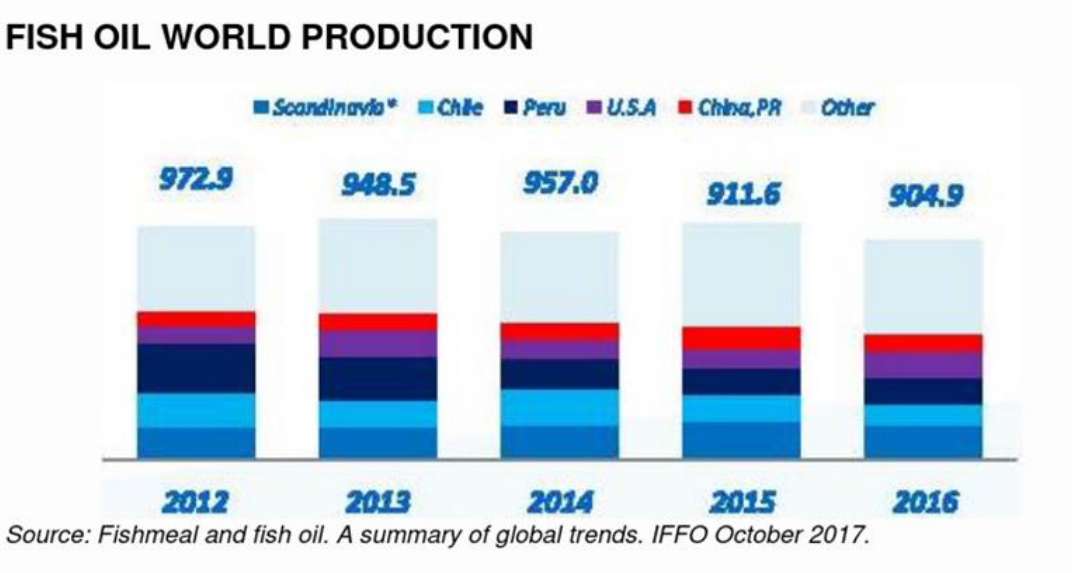
Fish oil



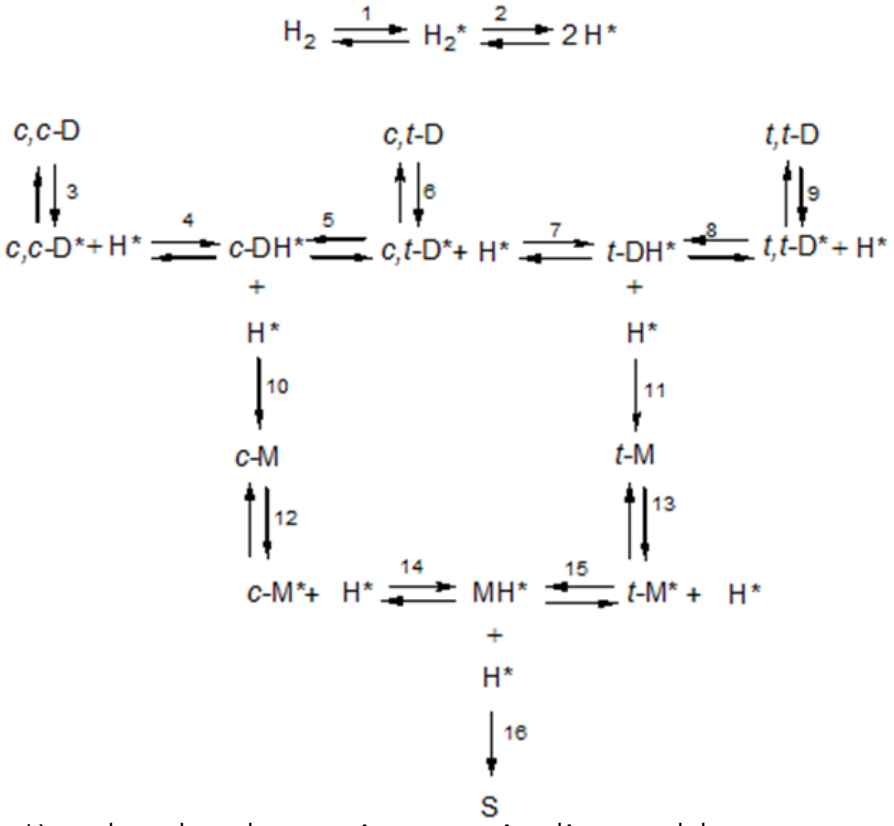


Source: Fishmeal and fish oil. A summary of global trends. IFFO October 2017.

In 1990 around 76 % of the fish oil was used as raw material in margarines (abandoned), now it is used mainly for aquaculture (together with fishmeal)

https://lipidlibrary.aocs.org/edible-oil-processing/marine-oils

Hydrogenation of edible oils

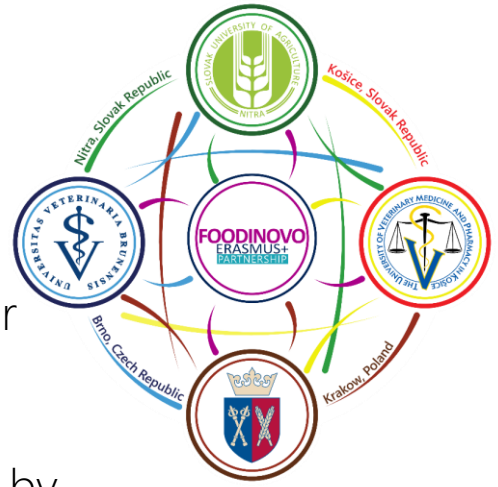


- Hydrogen is adsorbed onto the nickel surface (reaction 1) and dissociated into two hydrogen atoms (reaction 2).
- Fatty acids are adsorbed onto the nickel surface by their double bond or bonds (reactions 3,6,9)
  - In a first step, a hydrogen atom is added to double bond to form a half-hydrogenated intermediate (reactions (4,5,7,8).
  - If a second hydrogen atom is then added to this intermediate, the original double bond has been saturated (reactions 10,11) but because the first addition is reversible, the intermediate can also dissociate..
- Reversible reactions can result in an isomerisation that is both geometrical and positional

*) adsorbed species are indicated by an asterisk

https://lipidlibrary.aocs.org/edible-oil-processing/hydrogenation-mechanism

Aims of hydrogenation



- To convert a liquid oil into a solid fat. When solid fats of the right consistency are expensive or not available, hydrogenation, sometimes in combination with other processes such as interesterification or fractionation, may provide a way to produce the desired fat.
- To change the consistency of a fat. The melting point of a hydrogenated fat can be controlled by the degree of hydrogenation. Vegetable oils contain practically exclusively cis isomers of fatty acids. Hydrogenation will also convert some of the cis isomers into trans isomers, which give the triglycerides different melting characteristics. Furthermore, by using specific catalysts and/or hydrogenation conditions (such as temperature and hydrogen pressure), the composition of the fatty acids and the level of cis and trans isomers occurring at a particular iodine value (IV) can be controlled. Consequently, the melting behavior of the fat at a particular IV can be influenced by the processor.
- To improve oxidative stability of oils or fats. In general, saturated fatty acids are chemically more stable than unsaturated fatty acids. By converting unsaturated fatty acids to less unsaturated ones, the shelf life of the product will be improved and also the product may become more suitable for heavy-duty functions, such as frying.
- To broaden the availability of edible oils and fats. Whale oil and later fish oil are too ‘fishy’ for consumption. By hydrogenating these oils, palatable hardstocks were made available.

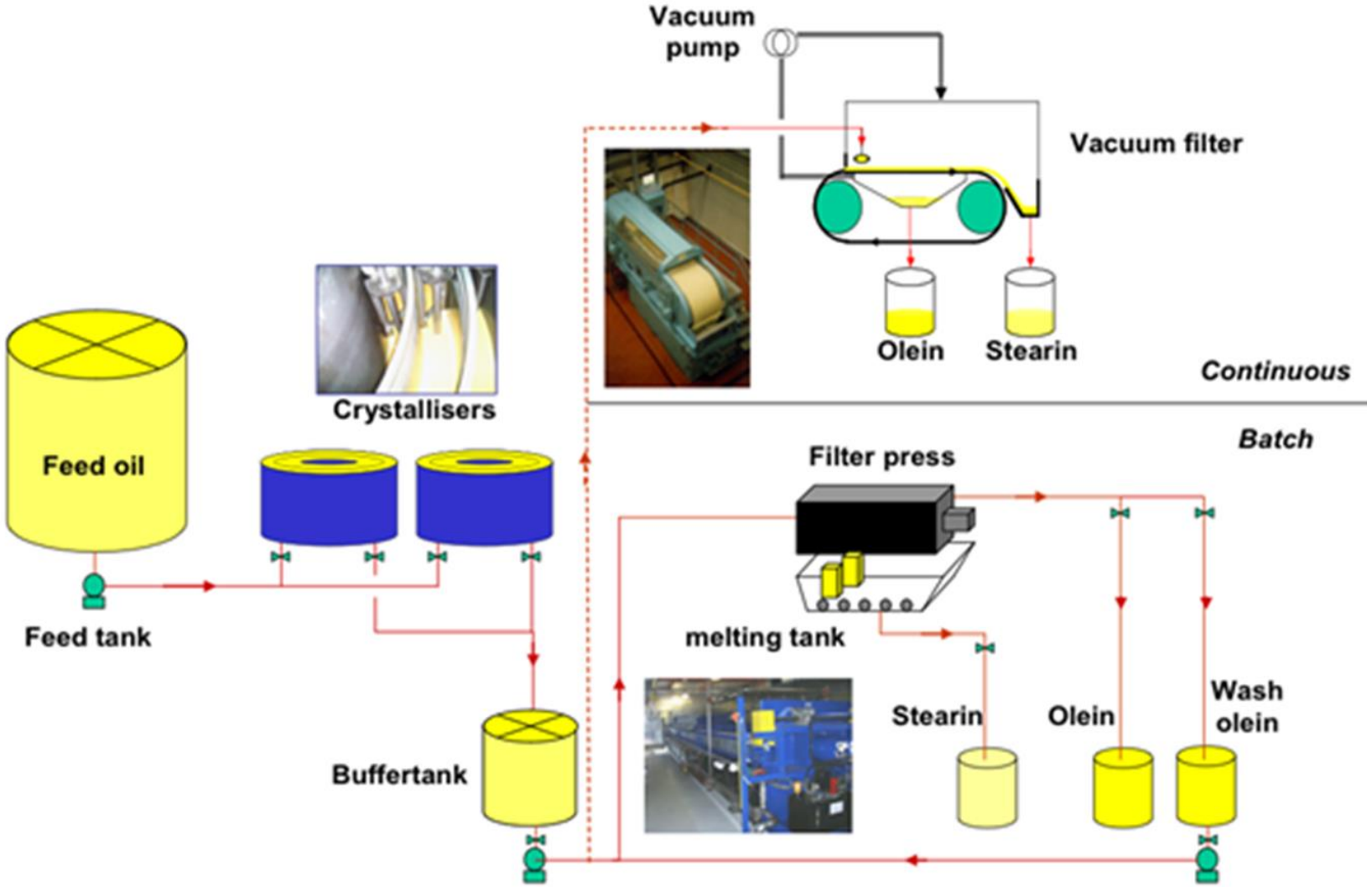
Interestrification of lipids

- Procedure to rearrange the composition of acids in triacylglycerols
- Alkaline catalyst – random distribution of acids
- Enzymatic processes:
- Control over the nature of the products
- Lipases may be specific for:
  - chain-length or double bond position of fatty acids
  - glycerol esters (mono-, di- or triacylglycerols)
- Milder conditions - less costly equipment
- Less waste and less effort is required to purify the product
- .

Fractionation of lipids

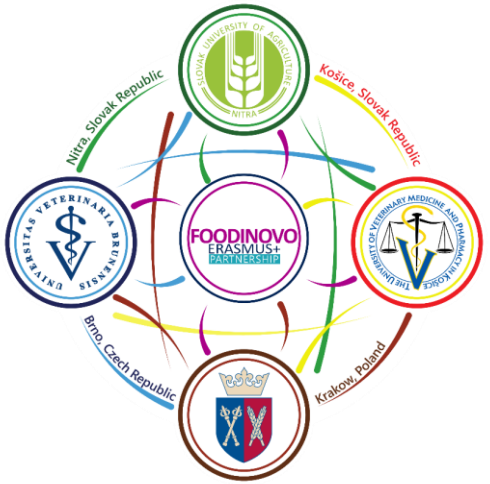
- Hippolyte Mège Mouriès (1817–1880): "Application for a patent of fifteen years for production of certain fats of animal origin"
- High temperature: fractional distillation
- Low temperature: winterization (dewaxing)
- Room temperature: supercritical extraction (high cost); urea complexation (unsuitable for triacylglycerols); membrane separation
- Fractional crystallization
  - Aqueous systems – detergent fractionation
  - Solvent (wet) fractionation (acetone, hexane, isopropyl alcohol)
  - Dry fractionation – crystallization from the melt (more saturated fats than in the case of winterization)

Fractionation of lipids



Dry fractionation process (https://lipidlibrary.aocs.org/edible-oil-processing/dry-fractionation

Specific Identification Tests for Fats and Oils:



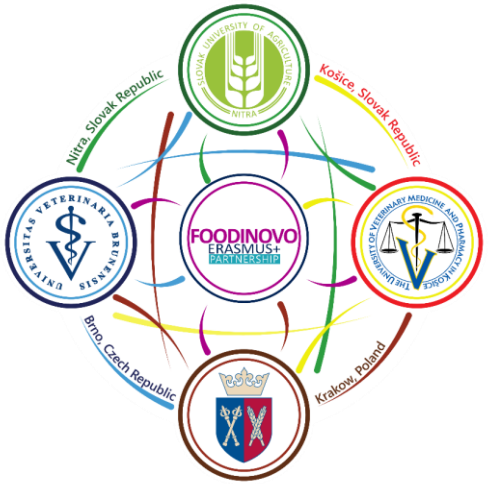
Butter: Reichert-Meissel index - content of volatile and water-soluble butyric acid (and other short chain fatty acids); >24 (ml of 0.1N KOH required to neutralise the soluble volatile fatty acids derived from 5 gm of fat).

Olive oil: Squalene content (7000 ppm) - very specific constituent of unsaponifiable part of olive oil (other oils 30-400 ppm).

Cottonseed oil: Halphen reaction detects presence of cyclopropenoic acids (malvalic and sterculic) which form a red color on heating with amyl alcohol and sulphur dissolved in CS_2.

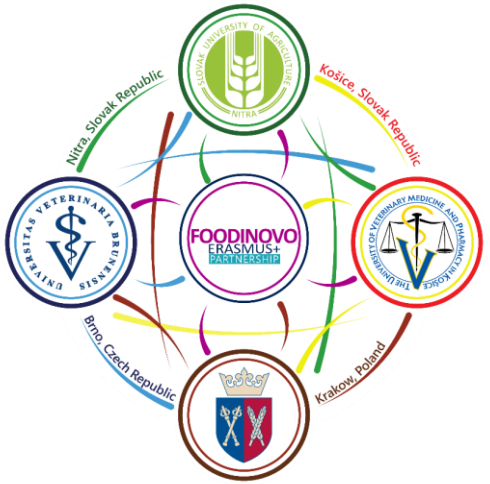
Sesame oil: Villavechia (or Baudouin) test: unsaponifiables of sesame oil form pink color with furfural (not feasible to detect refined sesame oil).

Adulteration detection



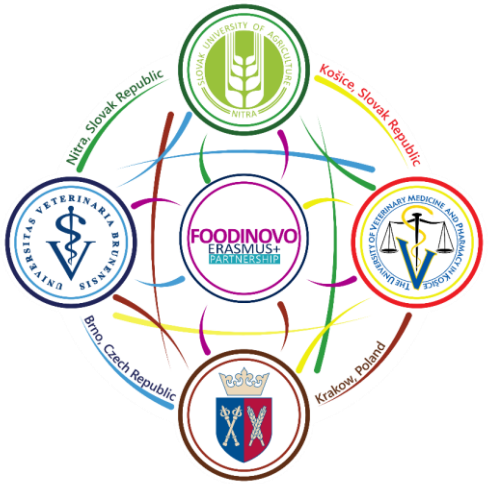
- Most expensive oils are most prone for adulteration with cheap ones:
  - Adulterated: (E)VOO, cocoa butter, argan oil, cold pressed oils
  - Adulterants: soy oil, sunflower oil, canola oil, corn oil, POO, chemically extracted oils
- Range of methods (goal: non-destructive, accurate, simple and cheap testing system)
  - voltametric e-tongue; electrochemical techniques, mass spectrometry, IR, chromatography, DNA-based
- Data processing techniques:
  - multivariate analyses: principal component analysis, soft independent modeling of class analogy, partial least square

Instrumental methods of lipid determination



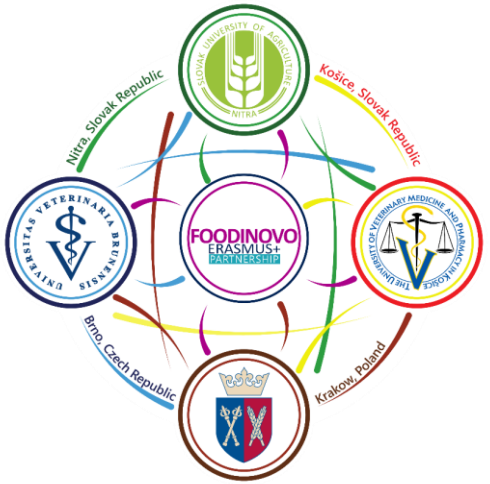
- Density measurements
- Ultrasonic methods
- Dielectric methods, conductometry
- Calorimetry
- Refractive index
- Spectroscopic methods
  - Turbidimetric/colorimetric
  - Infrared / Raman
  - Nuclear magnetic resonance (NMR)
  - X-Ray
  - Electron spin resonance (ESR)
- Mass spectroscopy

Extraction of Food Lipids



- Methods of Association of Official Analytical Chemists (AOAC)
- Multiple stages:
  - pretreatment of the sample e.g., drying, size reduction, hydrolysis;
  - homogenization of the tissue in the presence of a solvent;
  - separation of liquid (organic and aqueous) and solid phases;
  - removal of nonlipid contaminants;
  - removal of solvent and drying of the extract.
- Sample preparation for lipid analysis depends on the type of food and the nature of its lipids
- Immediate extraction is advisable (but not always possible)

Pretreatments

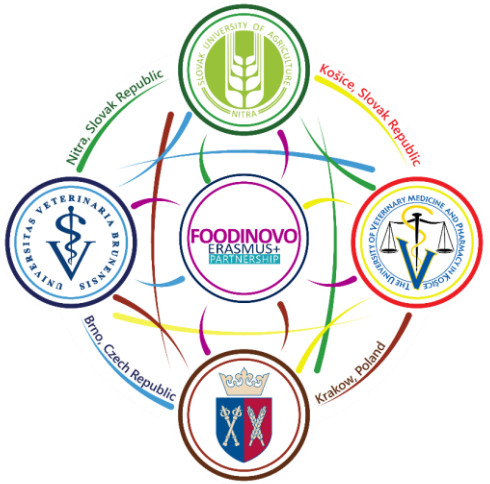


- Drying:
  - vacuum oven drying or lyophilization – higher temperatures may lead to lipid bounding to carbohydrates or proteins, remaining water reduces solvent's extraction capability
- Grinding/milling/homogenization
  - enhanced extraction due to higher surface area
- Hydrolysis
  - acid (usually 3–6 M HCl), alkali (ammonia), enzymes (proteases, carbohydrases; e.g., Clarase a mixture of α-amylase and protease)

Lipid extraction

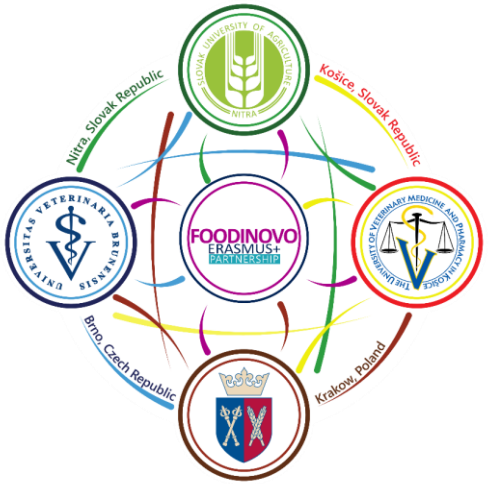
- Choice of organic solvent
  - Free lipids - light petroleum ether or diethyl ether
  - Bound lipids – more polar solvents, such as alkanols
  - Lipids covalently bound to polypeptides or polysaccharides could not be extracted without hydrolysis step
- Non-organic solvents
  - Supercritical fluid extraction (SFE)
- Extraction Without Solvents
  - Acid Digestion Methods
  - Detergent Method
  - **Physical Methods**
- Extract cleaning
  - main contaminants oil-soluble flavors, pigments, sugars, amino acids, short chain peptides, inorganic salts, and urea
  - water or a diluted KCl solution (0.88%, w/v)
  - chloroform–methanol (2:1, v/v)

Solid-phase extraction



- mid-1970s - quick and efficient sample preparation for lipid analysis
- Lipids in hydrophilic media are retained on the SPE column, while the nonlipid impurities are allowed to pass through
- Collection of lipids by organic solvents with low polarities
  - class separation possible by using multiple elution steps
- SPE cartridges
  - Reversed phase (C18- alkyl groups attached to microspheres of silica), eluent chloroform–methanol (1:2, v/v)
  - Normal phase (amino- (NH2), cyano- (CN) and silica (Si)
  - Ion-exchange - weak or strong cation and anion exchangers

Chromatographic Procedures for Lipid Characterization

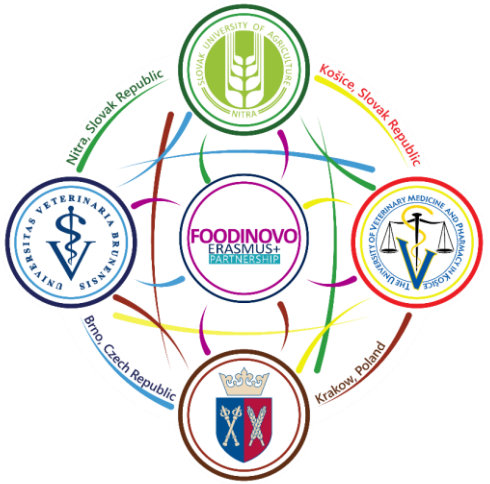


- Thin layer chromatography TLC
- Gas chromatography (GC),
- Supercritical fluid chromatography (SFC)
- Column chromatography
- Solid-liquid, liquid-liquid, ion-exchange
- High-performance liquid chromatography (HPLC)

GC

- Allows determination of overall FA composition (total trans FA, saturated FA (SFA), monounsaturated FA (MUFA), and PUFA)
- Harsh acid-digestion procedure - partial or complete destruction of acid-labile compounds – alternative - direct methylation of FA in food matrices
- Derivatization of fatty acids to increase their volatility
- Fatty acid methyl esters (FAME)
- Mono- and diacylglycerols have to be converted to trimethylsilyl (TMS) or tert-butyldimethylsilyl ethers (TBDMS)
- Gas phase:
- Nitrogen or helium (packed columns)
- Helium or hydrogen (capillary columns)
- Flame ionization detection (FID)
- May be combined with mass spectrometry (GC-MS)

TLC



- The earliest chromatographic method used for lipid assessment
- Stationary phase - silica gel
- Mobile phase - organic solvents such as chloroform, methanol; water and modifiers
- One-dimensional TLC - simple mixtures
- Two-dimensional TLC (2D-TLC) - complex mixtures
- Quantitative determination – densitometry combined with various developing agents
- Advantages: simplicity, high resolving power, and affordability,
- Limitations: low resolution, sensitivity and lipid recovery

HPLC

- Mild conditions – suitable for heat-sensitive components
- Hydrolysis or saponification for FFA analysis
- Column packaging – depends on partition mode:
- Normal phase (NP-LC) - separates lipid classes from the most hydrophobic to the most hydrophilic; silica, alumina and cyano columns
- Reverse phase (RP-LC) - separates individual fatty acids; lipids within the same class separate according to carbon chain length and quantity of double bonds; C18, C8 and octadecylsilyl columns
- Liquid phase – alcohols (methanol; 2-propanol), acetonitrile, hexane, chloroform and/or water (constant composition/gradient elution)
- UV detection (double bonds in carboxyl group 203–210 nm - avoid chloroform and methanol-based solvents)
- Fluorescence detection: 9-anthryl-diazomethane (ADAM) derivatives
- Refractive index (RI) - sensitive to temperature fluctuations, unsuitable for solvent gradients
- Evaporative light-scattering detection (ELSD) and charged aerosol detection (CAD).

SFC

- Carbon dioxide compressed at a temperature and pressure above its critical point does not liquify but forms a dense gas - mobile phase for LC
- Supercritical fluid extraction (SFE) - lipid extraction method, combined with ELSD also allows quantification (instead of gravimetric analysis)
- Temperature required for SC-CO2 much lower than for GC; pressure - HPLC
- Range of columns: HPLC and GC capillaries

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