Basic oil chemistry for producers of indigenous oils

A guide to testing, analysis and quality production

ABioSA TRAINING MANUAL

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Contents

This training manual is part of a series of knowledge products produced by ABioSA. These knowledge products and other biotrade resources can be found at www.abs-biotrade.info/projects/abiosa/resources

A glossary of biotrade terms can be found at www.abs-biotrade.info/resources

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Glossary

- **Affective testing** - also referred to as hedonic or ranking tests, is a process in which people rank products against their preferences
- **Aliphatic** - refers to chains made up of carbon atoms
- **Alkali refining –** the process by which alkali is used to neutralise free fatty acids
- **Antioxidants** - substances that can prevent oxidation or damage to oils
- **Anisidine Value** (AV) - used to measure secondary oxidative deterioration. It is a measure of the aldehyde levels in an oil or fat, in particular those that are unsaturated. AV should be combined with the Peroxide Value for a complete picture of oxidative deterioration over time, particularly for refined oils or oils with high levels of unsaturation.
- **BHA** (Butylated hydroxyanisole) - synthetic antioxidant
- **BHT** (Butylated hydroxytoluene) - synthetic antioxidant
- **Carboxylic acid** - organic acid containing a carboxyl group attached to an R group
- **Carotenoids** - plant pigments with antioxidant activity and responsible for bright red, yellow and orange hues of oil
- **Carrier oils** - vegetable oils used to dilute essential oils used in aromatherapy
- **Centrifugal process** - the use of centrifugal force to separate particles from a solution
- **Certificate of Analysis** - document reporting the results of analysis obtained during laboratory analysis
- **Chemical refining** - a process that involves degumming, alkali neutralisation, dewaxing/winterisation, bleaching and deodorisation. Free fatty acids are removed by an alkali.
- **Physical refining** - a process that involves degumming, neutralisation, dewaxing/winterisation, bleaching and deodorisation. Free fatty acids are removed by distillation.
- **Chlorophyll** - green pigments found in plants
- ***Cis*** - a *cis* configuration means that the two hydrogen atoms adjacent to a double bond stick out on the same side of the chain
- ***Codex Alimentarius*** - is a collection of internationally recognised standards, codes of practice, guidelines, and other recommendations relating to foods, food production and food safety
- **Codex Commission** - established in 1961 by the Food and Agriculture Organisation of the United Nations, the Commission developed and maintains the *Codex Alimentarius*
- **Cold pressing** - the process to extract oil by screw pressing at a temperature no higher than 49°C
- **Commodity oils** - oils produced in large volumes for everyday use
- **Degumming** - the process of hydrating phosphatides in an oil by adding water followed by centrifugation
- **Deodorisation** - the last step of refining, using a steam-distillation process to remove free fatty acids and volatile components in crude edible oil
- **Distillation** - removal or separation of compounds from a liquid mixture by selective boiling and condensation
- **Enzymes** - proteins acting as biological catalysts to accelerate reactions. Enzymes can speed up formation and breaking of bonds.
- **Essential oils** (often referred to as aromatic oils) - concentrated liquid containing volatile chemical components from plants
- **Fats and oils** - lipids which are solid at room temperature are called fats, and lipids which are liquid at room temperature are referred to as oils

- **Fatty acid profile** - can consist of a combination of the following:
  - Short-chain fatty acids have five or fewer carbons
  - Medium-chain fatty acids have six to 12 carbons
  - Long-chain fatty acids have 13 to 21 carbons
  - Very long chain fatty acids have aliphatic tails of 22 or more carbons

- **Free Fatty Acid Value** (FFAV) - indication of the amount of fatty acids which have been separated from the glycerol backbone

- **Free radicals** - unstable atoms that can damage cells

- **Functional properties** - useful effects of components in oil

- **Gas chromatograph** (GC) - a common type of chromatography (analysis) used to separate compounds

- **Glycerol** - backbone of triglyceride (fat/lipid/oil molecule)
  - three fatty acids are connected to a glycerol backbone to form a triglyceride (fat/lipid/oil molecule)

- **Hydrogenation** - a chemical reaction used to break double bonds and convert a liquid oil to a solid fat

- **Hydrolytic deterioration** - caused by moisture, heat and enzymes

- **Hydrophobic** - compounds repelled by water

- **Hydroperoxides** - compounds formed during the process of oxidation when the double bonds in oil are exposed to oxygen

- **Initiation, propagation and termination** - the three steps of oil oxidation

- **Iodine Value** (IV) - the degree of unsaturation of a fat or oil

- **Lipid oxidation** - the attack of oil by oxygen, leading to the development of odours and a decrease in the nutritional value of oil

- **Macro-components** (triglycerides) - lipid molecules consisting of one glycerol molecule and three fatty acids

- **Malaxeur** - instrument used to knead raw materials to ensure that larger particles are broken down and that the product is well mixed together

- **Mechanical pressing** - process where oil is forced from oilseed either by using a screw press or hydraulic press

- **Micro-components** (unsaponifiable matter) - part of the oil that cannot be saponified (made into soap), but which is soluble in oil including waxes, vitamins and sterols

- **Mono-unsaturation** - if a fatty acid contains only one double bond, it is mono-unsaturated

- **Neutralisation** - the process of removing free fatty acids from crude oils by using caustic soda or lime

- **Nitrogen blanketing** - the process of introducing nitrogen to a storage tank to counter the effects of oxygen on the material being stored

- **Oils** - triglycerides which are in a liquid state at room temperature

- **Omega** - the position of a carbon double bond is indicated with the omega sign

- **Oxidative deterioration** - reaction of fats and oils with molecular oxygen leading to off-flavours that are generally called rancid

- **Oxidative stability** - an important quality characteristic of all oils, governed by the inherent composition of the oil, namely the fatty acid composition or macro-components, as well as the content of micro-components including tocopherols, polyphenols, carotenoids and chlorophyll

- **Peroxide Value** - is an indication of primary oxidative deterioration and a measure of hydroperoxide levels

- **Peroxy radicals** - are compounds formed during lipid oxidation and measured by Peroxide Value

- **Polyphenol** - compounds naturally found in foods with various antioxidant and health benefits

- **Plant sterols** - a group of substances made in plants and found in the highest amounts in foods like vegetable oils, nuts and seeds. Plant sterols have medicinal purposes and are most commonly used for lowering cholesterol.

- **Poly-unsaturation** - if the fatty acid has more than one double bond it is poly-unsaturated. Oils containing mainly poly-unsaturated fatty acids are referred to as poly-unsaturated oils.

- **Prooxidants** - chemicals that induce oxidative stress either by generating reactive compounds or by inhibiting antioxidant systems

- **Refractive index** - measures the bending of light when passing from one medium to another. It is an indication of purity as well as unsaturation of the oil.

- **Relative density**, also called **specific gravity** - the ratio of the density of a substance to the density of a reference material

- **Saponification Value** (SV) - indicates the suitability of an oil for soap making. It is a reflection of the chain length of the fatty acids in the fat or oil, and is a measure of the average molecular weight of all the fatty acids present.

- **Seed oils** - vegetable oils obtained from the seed of a plant rather than a fruit

- **Sensory evaluation** - a smelling and tasting test

- **Steam refining** - the process of removing free fatty acids with distillation, also called physical refining

- **Supercritical carbon dioxide extraction** - extraction of oil using supercritical carbon dioxide as a solvent

- **Supercritical state** - when a molecule is in a supercritical state, it has both the properties of a gas and a liquid

- **TBHQ** (Tertiary-butyl hydroquinone) - synthetic antioxidant used to stabilise vegetable oils

- **Tocopherols** - a class of organic chemical compounds, many of which have Vitamin E activity

- **Trans fatty acid** - an unsaturated fatty acid molecule that contains a trans double bond, making a molecule less kinked compared to fatty acids with *cis* double bonds. A trans configuration means that the two hydrogen atoms adjacent to a double bond are located on opposite sides of the chain.

- **Triglycerides** - fat/lipid/oil molecules consisting of one glycerol molecule and three fatty acids

- **Unsaturated fatty acids** - fatty acids containing double bonds

- **Vegetable oils** - triglycerides extracted from seeds or fruit

- **Wijs method** - the test used to determine Iodine Value

- **Unsaponifiable matter** - includes substances frequently found dissolved in fats and oils which cannot be converted to soap, but which are soluble in ordinary fat and oil solvents. They usually consist of waxes and antioxidants.

- **Winterising** - is the process in oil refining where high-melting components like waxes are removed

Summary

The aim of this ABioSA training manual is to provide vegetable oil producers with information that helps to improve their production quality. It should enable them to decide which analysis to request, and then independently interpret the resulting Certificate of Analysis. They should also be able to determine whether further processing of their product is necessary.

The manual will supply the oil producer with the following knowledge:

- Basic components of oil
- The effects of external and inherent factors on oil quality
- Analytical methods
- Interpreting a Certificate of Analysis
- Available extraction and processing techniques

Oil is produced in South Africa from many indigenous and agricultural species including (clockwise from top left) Kalahari Melon (x2), Baobab, Olives, Sunflower and Trichilia Emetica

Picture Credit: *ABioSA/PhytoTrade/Proof Africa/Jonathon Rees*

Oil sources, production and uses

Picture Credit: *Bio-Innovation Africa/Jonathon Rees*

Vegetable oils are used for food or cosmetics. Commodity oils such as sunflower, palm, soybean and canola oil are generally used in the food sector, but also for cosmetic applications.

Speciality oils, including indigenous oils from southern Africa such as Marula, Kalahari melon, Ximenia, Baobab and Mafura are generally used as cosmetic ingredients.

Vegetable oils are valued for their unique fatty acid profile and micro-components including tocopherols, carotenoids and other antioxidants. Antimicrobial, moisturising and anti-inflammatory actions are some of the properties attributed to these compounds; and are projected to fuel demand and market growth. Consumer interest in organic and natural beauty products is expected to further boost demand.

Companies in the cosmetic oil market are introducing a variety of cold-pressed oils as a major ingredient in hair and facial products including lipsticks, lip balms, moisturisers, and serums. There is also increasing demand for hair and skin products containing vegetable oils which help cure problems like dandruff, dry scalp and dry skin.

The **global cosmetic oil market** was valued at US$50.58 billion in 2018 and is expected to register a compound annual growth rate of 5.2% from 2019 to 2025.

Sources of fats and oils

It is important to distinguish between vegetable oils (also referred to as fixed oils, fatty oils, carrier oils or seed oils) and essential oils (often referred to as aromatic oils), as the terms are often used incorrectly. This training manual is focused on vegetable oils.

Vegetable oil

Vegetable oil refers to any oil that originates from plant sources and has an oily texture consisting of triglycerides. The properties of a vegetable oil depend on its source and composition. Vegetable oils include, for example, olive, palm, coconut, soybean, sunflower, canola, Marula, Baobab and Ximenia. Vegetable oils do not evaporate when exposed to air. Vegetable oils are generally extracted using cold pressing, centrifugation or using solvents, and are used as a carrier oil for essential oils.

Essential oil

An essential oil is a concentrated hydrophobic liquid containing volatile chemical compounds from plants. Essential oils are also known as volatile oils because they evaporate when exposed to air.

Essential oils are extracted by means of distillation. Essential oils dissolve in vegetable oils, which are used as a carrier oil.

Soap can be produced from Marula oil.

Picture Credit: *Bio-Innovation Africa/Jonathon Rees*

Raw and processed products that contain oil

- Fruit e.g. olives, avocados
- Oilseeds e.g. sunflower seeds, macadamia nuts
- Fish & meat e.g. lard, tallow, fish
- Micro-organisms e.g. algae and fungi
- Dairy e.g. butter and cream

General uses of fats and oils

- Food industry ingredient or for frying
- Health supplements
- Soap
- Cosmetics
- Medical applications (e.g. triglycerides for tube feeding)
- Biodiesel
- Solvents
- Paints, flooring (linoleum floors)

Oil extraction methods

Mechanical pressing

There are two types of mechanical pressing. Hydraulic pressing is generally used for oilseeds with a high fat content and softer consistency, such as the kernels in a marula pip.

Screw pressing is used when seeds are harder and the screw pressing mechanism can grip the raw material, as in the case of Baobab and Kalahari melon seeds.

Preparing Marula kernels for screw pressing.

Picture Credit: *Bio-Innovation Africa/Jonathon Rees*

Solvent extraction

Solvent extraction is generally used to remove residual oil from the oilcake remaining after screw press extraction. This method is more productive than screw pressing and removes oil more effectively. It is very important that pure solvents are used as contaminated solvents can leave a residue in the oil with detrimental health effects.

Wet extraction

Wet extraction is used when a product has a high moisture content, such as olives or avocados. The whole fruit is broken into small pieces by means of a hammer mill or malaxeur, and the oil is then extracted by a centrifugal process. Enzymes are often used to aid in the breakdown of cell walls during wet oil extraction. This makes the oil in the cells more available.

Supercritical carbon dioxide extraction

Supercritical carbon dioxide extraction is one of the available green technologies that replaces the traditional organic solvent. It uses carbon dioxide at a supercritical temperature and pressure. When a molecule is in a supercritical state, it has properties of both a liquid and a gas, with excellent solvent properties. Supercritical carbon dioxide extraction is a common method to separate various components from a plant as it produces a pure, clean and safe product.

Compared with traditional solvent extraction, it uses supercritical fluid to provide a broad range of useful properties. It eliminates the use of organic solvents, which reduces the problems of their storage, disposal, and environmental concerns. Supercritical carbon dioxide extraction is very expensive and not often used.

Close up of kernels in a Marula seed. This is the source of Marula oil.

Picture Credit: *Bio-Innovation Africa/Jonathon Rees*

Basic oil chemistry

What are fats and oils?

An oil is a fat in a liquid state. It is important to understand their structure and characteristics in order to recognise how they will react under different conditions.

Oils and fats consist of macro-components (triglycerides) and micro-components (unsaponifiable matter). The macro-components generally make up around 99% of the fat or oil. The other 1% is micro-components which dissolve. This can, however, vary for different oil types (Shea butter, for example, has an unsaponifiable matter content of 16%).

Although present in very low levels, the unsaponifiable matter contains powerful antioxidants and functional properties which can distinguish one oil from another. The unsaponifiable matter contains many micro-components including tocopherols, sterols, carotenoids, polyphenols, chlorophyll and waxes.

Triglycerides are the main component in fats and oils. The physical properties of an oil are dependent on the structure of these components.

A triglyceride consists of glycerol and three fatty acids (*Figure 1*). It is the fatty acids that give the functionality to oils. Most naturally occurring fatty acids have an unbranched chain of an even number of carbon atoms, from four to 30.

Short-chain fatty acids have five or fewer carbons. Medium-chain fatty acids have six to 12 carbons. Long-chain fatty acids have 13 to 21 carbons; and very long chain fatty acids have aliphatic tails of 22 or more carbons.

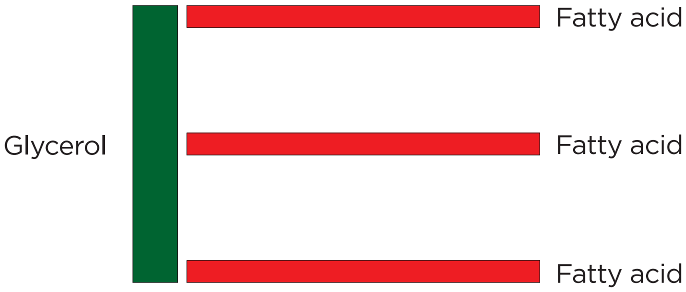


Figure 1. *A triglyceride consists of glycerol and three fatty acids*

Solid and liquid fats

A fatty acid is a carboxylic acid with a long aliphatic chain, which is either saturated or unsaturated. Although the terms oils and fats are often used interchangeably, they are usually used to distinguish triglycerides in the liquid state at ambient temperatures (oils) from those in the solid state (fats).

A saturated fat has single bonds between its carbon atoms. An unsaturated fat has one or more double bonds between its carbon atoms. The higher the level of saturation in a fat, the harder its consistency at room temperature. Oleic acid (the main fatty acid in olive, avocado, macadamia and Marula oil) is an example of an unsaturated fatty acid.

Most unsaturated fats are liquid at room temperature and are called oils. When oils are solid at room temperature, they are called fats. The *cis* double bond causes a bend or a kink in the carbon chain. This prevents the fatty acids from packing tightly into a solid, keeping them liquid at room temperature (*Figure 2*).

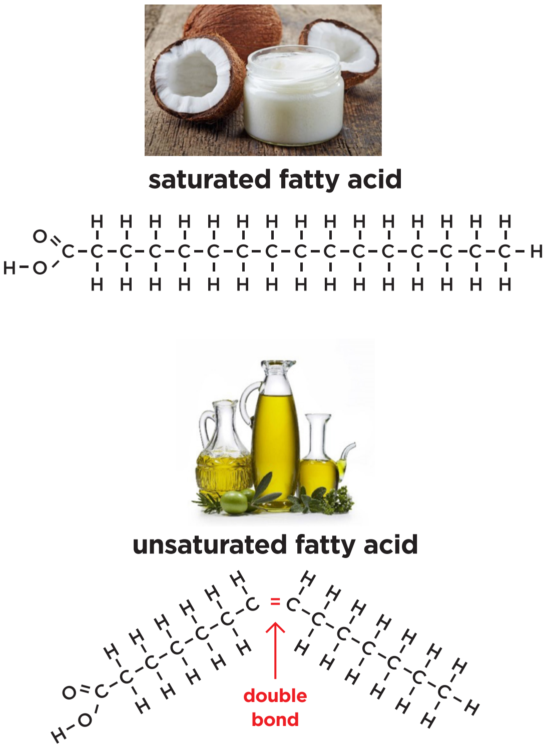


Figure 2. *Saturated fatty acids make fats hard or solid, while the kink of unsaturated fatty acids makes them liquid*

Mono-unsaturation and poly-unsaturation

If a fatty acid contains only one double bond, it is **mono-unsaturated**. If the carbon chain has more than one double bond it is **poly-unsaturated**. Oils containing mainly mono-unsaturated fatty acids are referred to as mono-unsaturated oils. Oils containing mainly poly-unsaturated fatty acids are referred to as poly-unsaturated oils.

Naming fatty acids

All fatty acids consist of a carbon chain. They are named according to the number of carbon atoms in the chain as well as the amount of double bonds present (*Figure 3*). The shortest descriptions of fatty acids include only the number of carbon atoms and double bonds in them (e.g. C18:0).

The following example illustrates the naming of fatty acids:

C18:0 will have 18 carbon atoms and no double bonds

C18:1 will have 18 carbons and one double bond (mono-unsaturated)

C18:2 will have 18 carbon atoms and two double bonds (poly-unsaturated)

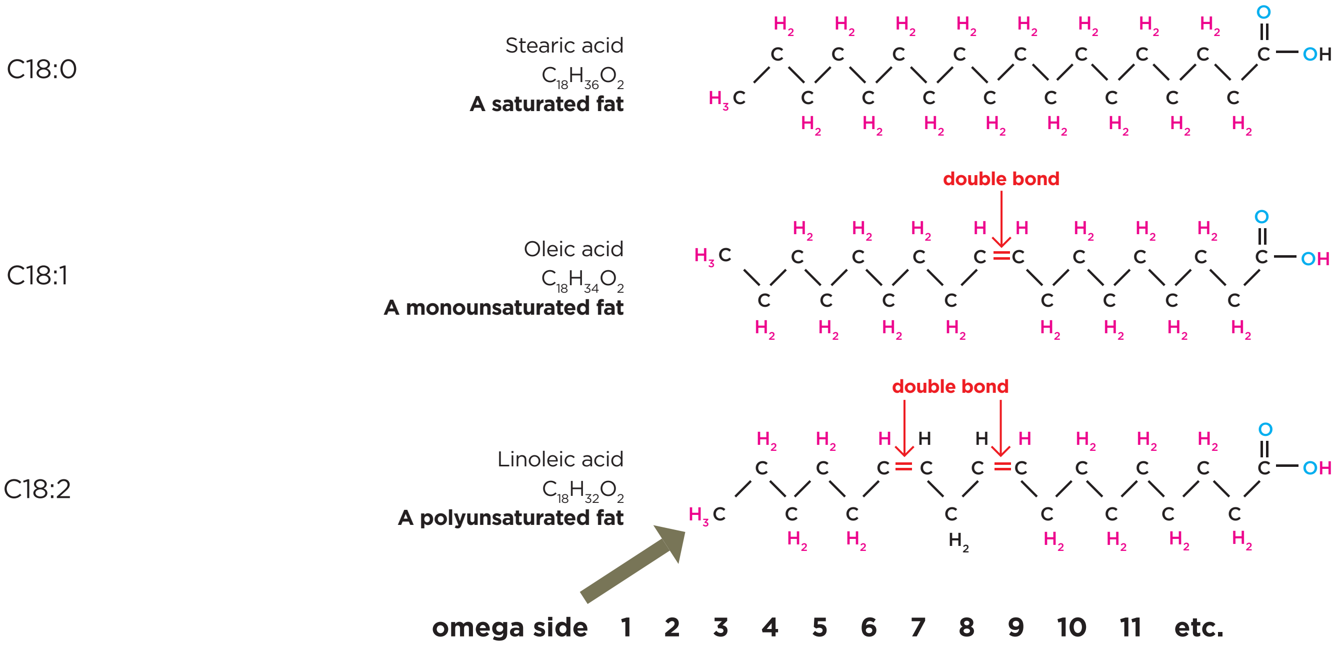


Figure 3. *Naming of fatty acids*

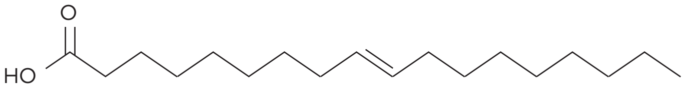
What does Omega mean in naming of fatty acids

The position of the double bond is indicated with the Omega sign. The Omega (ω) nomenclature means that carbons are counted from the methyl (Omega) end of the fatty acid chain (*Figure 3*). If the double bond occurs on the 6th position from the Omega side of the fatty acid chain, for example, it will be referred to as an Omega 6 fatty acid. If most of the fatty acids present in an oil have double bonds on the 6th position, the oil is usually referred to as an Omega 6 oil. The same is applicable to Omega 3 and Omega 9 oils, which are often marketed for their health benefits.

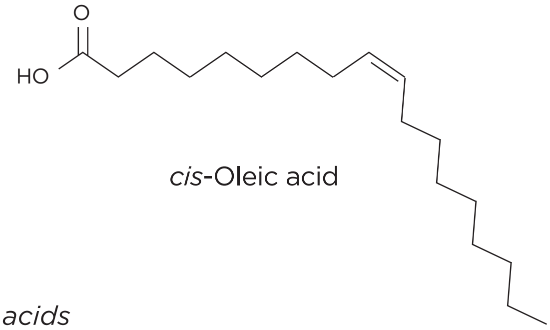
Cis *and trans fatty acids*

Cis fatty acids are the natural form of unsaturated fatty acids. A trans fatty acid (commonly shortened to trans fat) is an unsaturated fatty acid molecule that contains a trans double bond, which makes the molecule less kinked in comparison to fatty acids with *cis* double bonds.

These bonds are characteristically produced during industrial hydrogenation of plant oils, but can also form during frying at high temperatures. Research suggests that increasing amounts of trans fats are, for reasons not well understood, more correlated with circulatory diseases such as atherosclerosis and coronary heart disease, than the same amount of non-trans fats.



trans-Oleic acid



cis-Oleic acid

Figure 4. *Trans fatty acids are less kinked than* cis *fatty acids*

Oil quality

An important quality characteristic of all oils is their oxidative stability. This is governed by the inherent composition of the oil, namely the fatty acid composition or macro-components, as well as the content of micro-components, including tocopherols, polyphenols, carotenoids and chlorophyll. According to Gunstone (1996), the ratio of oxidation of saturated, monounsaturated and polyunsaturated fatty acids is 1:12:25, making polyunsaturated oils the least stable, and saturated fats the most stable.

Micro-components act as antioxidants, protecting the oil against free radicals and therefore oxidative deterioration. The stability of the oil is also dependant on the quality of the oilseed or fruit before oil extraction, conditions under which oil is extracted, and storage after extraction. It is imperative that oil producers understand the practical principles which ensure the production of high-quality oil.

Deterioration of oil

Lipid oxidation is one of the major causes of food spoilage and is of great economic concern to the food industry. The main effect of lipid oxidation is the development of odours and a decrease in the nutritional value of food. The shelf life or oxidative stability of fats and oils is a priority for fats and oil manufacturers. The shelf life is also linked to the preservation of the specific antioxidants of the oil.

To understand oxidative stability, it is important to understand the composition of edible oil, which consists mainly of triglycerides comprised of three fatty acids (which can be saturated, mono- or polyunsaturated), and attached to a glycerol backbone (*Figure 5*).

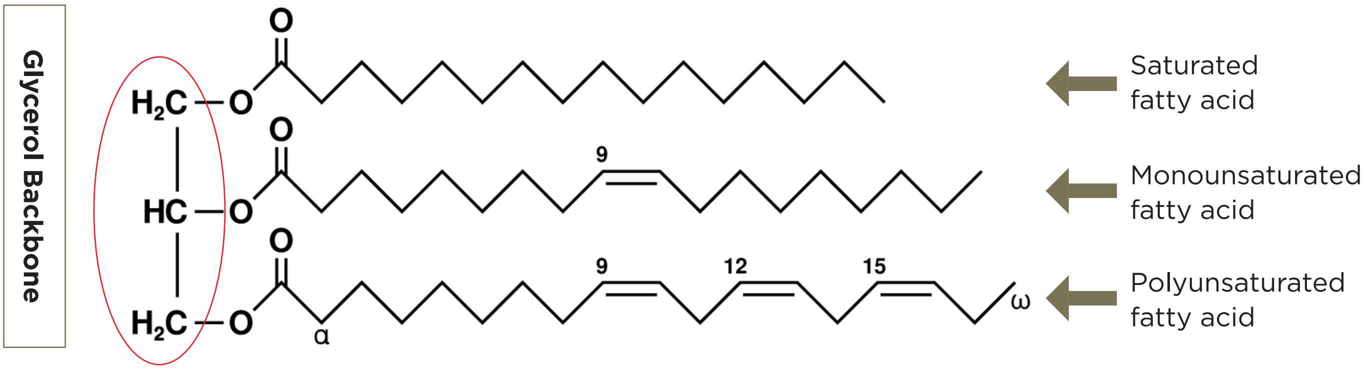


Figure 5. *Triglyceride structure showing different fatty acids connected to glycerol*

Lipid oxidation occurs when the structure of the oil molecule is damaged or altered. The deterioration of oil can be hydrolytic or oxidative. Deterioration of oil is caused by:

Factors inherent in the oil
- Chain length
- Amount of double bonds
- Antioxidant content

External Factors
- Light
- Heat
- Oxygen
- Metal ions acting as prooxidants
- Enzymes
- Moisture

Hydrolytic deterioration of oil

Hydrolytic deterioration is mainly encountered before oil is extracted from the oilseed or fruit. Triglycerides are hydrolysed or split from the glycerol backbone and free fatty acids are released (*Figure 6*). Hydrolytic deterioration is caused by the combinations of moisture, heat and the presence of enzymes such as lipases.

This combination occurs when moisture is not completely removed from oilseeds or fruit is overripe before pressing. The moisture in the seeds enables enzymes to remain active. Enzymes can originate from the fruit or other plant parts, or from micro-organisms present on the fruit or seed. This is particularly a concern in the Marula oil industry as the Marula nut is susceptible to fungal growth due to the presence of moisture and sugar in the moist fruit residue.

The effect of hydrolytic deterioration is even more pronounced when working with oils extracted from fruit, as is seen in the olive oil, avocado oil and palm oil industries. Olive oil, for example, is graded with regards to free fatty acid content as it gives a good indication of the sensory and stability characteristics of the oil.

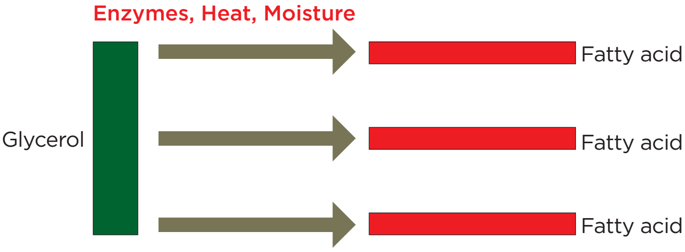


Figure 6. *Hydrolytic deterioration occurs when fatty acids are split from the glycerol backbone and free fatty acids are released. This is caused by combinations of moisture, heat and the presence of enzymes.*

Free Fatty Acid Value (FFAV)

Hydrolytic deterioration is measured with an Acid Value test or FFAV test. The Acid Value and FFAV express the same property of the oil. They are an indication of the amount of potassium hydroxide needed to neutralise the acidity caused when fatty acids are separated from the glycerol backbone. To express the free fatty acid content, the Acid Value (mg KOH/1g oil) is divided by 1.99 and expressed as 'g' oleic acid/100g oil. According to the general guidelines prescribed by the *Codex Alimentarius*, FFAVs of up to 0.3g oleic acid/100g oil are generally allowed for refined oils, while values of up to 2g oleic acid/100g oil are allowed for crude oils.

Free fatty acids are prooxidants and will cause the formation of more free fatty acids. The first factor determining the quality of the oil is the quality of fruit or oilseeds which are used for extraction. If the fruit, nuts or seeds are overripe, bruised or have a high amount of fungal growth, the enzyme activity will be elevated and the free fatty acid content will rise. Kalahari melon seeds, for example, have to be dried very well, to eliminate moisture which enables enzymes to remain active and in turn can increase the FFAV. Although free fatty acids can be removed by refining, an oil which has been damaged will have a reduced shelf-life even after refining.

Oxidative deterioration of oil

Oxygen and elevated temperatures are the main factors influencing the oxidative deterioration of lipids. Other factors include packaging, moisture, light; and unsaturation, chain length of lipids, metal ions, and antioxidants.

It is important to use containers that are not permeable by air when oil is packaged or sent to the laboratory. The headspace should be eliminated or minimised and if possible, the container should be filled to the top, to reduce the amount of oxygen which comes into contact with the oil as this will cause oxidation.

Longer chain fatty acids are more prone to oxidation. Fatty acids with more double bonds are also more susceptible to oxidation as the double bonds are where fatty acids are attacked by oxygen.

Lastly, it is important to eliminate metal pipes and storage units from the system, as metal ions get dissolved into the oil and cause its deterioration. To prevent contamination of oil by heavy metals (especially iron and copper), it is important to use stainless steel tanks, pipes and pumps in the extraction and storage systems of any oil extraction plant.

Oxidative deterioration involves reactions of the double bonds in fatty acids and can be divided into initiation, propagation and termination (*Figure 7*). During initiation, oxygen reacts with unsaturated fatty acids to form highly reactive compounds called free radicals. This step cannot be stopped by additives so it is important to remove all oxygen as far as possible from oil production and storage, as it will start oxidative deterioration. A nitrogen blanket can be applied to minimise contact with oxygen. This can be done by flushing the remaining headspace of the container with nitrogen. Nitrogen is heavier than air, so will form a heavy layer on the surface of the oil. Even more effective is bubbling nitrogen through the oil before storage, to remove any dissolved oxygen. It is important to seal the container immediately after the nitrogen treatment, as nitrogen will diffuse into the air if not contained.

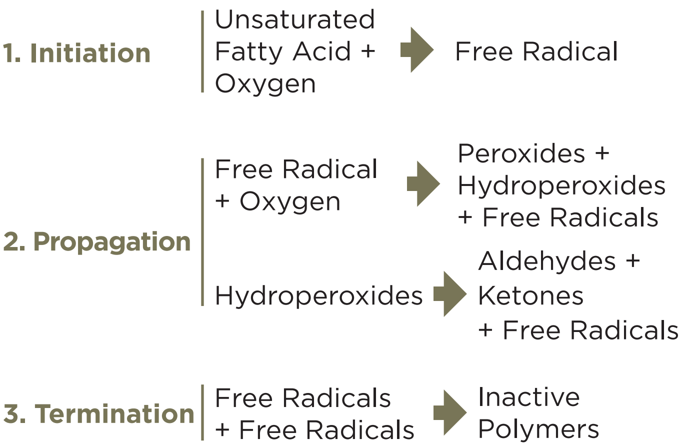


Figure 7*. Initiation, propagation and termination steps of oxidation*

During propagation, oxygen reacts with free radicals to form peroxy radicals which react with other unsaturated fatty acids to form hydroperoxides. This step will continue until oxygen is depleted or a reaction with a stable antioxidant occurs. Degradation of hydroperoxides is accelerated by prooxidants like heavy metal ions and heat, and is considered to be a starting point for the formation of volatile reaction products.

The latter are usually powerfully odorous compounds (mainly ketones, aldehydes and alcohols), perceived as rancidity by the consumer.

The last step of oxidation is termination, where the free radicals eventually react with each other to form stable end products.

Antioxidants are often added to oil to prevent the breakdown of hydroperoxides by inhibiting the propagation process, and prevent rancidity by breaking the oxidation chain. These chain-breaking antioxidants include tocopherols, polyphenols, carotenoids and flavonoids as well as the synthetic antioxidants BHA (Butylated hydroxyanisole), BHT (Butylated hydroxytoluene) and TBHQ (Tertiary-butyl hydroquinone).

Rosemary and oregano extracts have also effectively been used as antioxidants for oil.

Peroxide Value (PV) and Anisidine Value (AV)
Tests for oxidative deterioration usually include Peroxide Value (PV) and Anisidine Value (AV). PV is an indication of primary oxidative deterioration and measures hydroperoxide levels, while the AV is used to measure secondary oxidative deterioration.

During oxidative deterioration, the PV increases to a maximum and then decreases until all available hydroperoxides have been broken down to form secondary odorous oxidation products measured by the AV.

PV alone can be deceptive as oxidation might be advanced and still show a low PV. Therefore the AV and PV must be measured together to get a complete picture.

According to *Codex Alimentarius*, a refined and crude oil can have a maximum PV of 10meq/kg (milli-equivalents per kilogram oil) oil and 15meq/kg oil, respectively. This is on the high end of the spectrum and commercially a PV of 2meq/kg oil and 4meq/kg oil is acceptable.

The significance of the AV should not be underestimated, as the PV can be deceptively low, while the AV is already elevated. It is also difficult to pinpoint a maximum PV because, although peroxides are constantly being formed, they are also constantly being broken down to form secondary products. The AV is therefore a measure of the number of peroxide reactions that took place.

In general, AVs above 6 mmol/kg indicate rancidity, while values above 4 mmol/kg oil indicate reduced shelf life. Standards Malaysia (Malaysian Standard 814:2007) requires that refined palm oil should have an AV of less than 4 mmol/kg (milli-moles per kg oil). This is a typical decision point.

At the end of the shelf life of sunflower oil, when the end of the oxidative deterioration chain has been reached as determined by sensory evaluation, the oil has an AV of 21 to 45 mmol/kg oil. Rancidity can usually be detected at an AV of 10mmol/kg oil.

The value of sensory evaluation (smelling and tasting of oil) in the determination of rancidity in vegetable oils should not be underestimated.

Sensory tests may be divided into three groups based on the type of information they provide. The three types are discrimination, descriptive, and affective. The selection of the appropriate test should be based on clearly defined objectives for the project.

The triangle test is a discriminative method with many uses in sensory science, including gauging if an overall difference is present between two products. This can be done by an untrained panel.

Affective testing can also be done by an untrained panel. In affective testing, taste is ranked from like to dislike and it is often used to test a new product on consumers. A trained panel will taste for specific good and bad flavours, and is also expected to rank the intensity of each flavour. This test is often used to determine rancidity (oxidative deterioration in oil).

The FFAV, PV and AV can tell how the oilseeds or fruit were harvested and stored and the efficiency of refining. Should the free fatty acids be elevated, the problem is most likely with a raw material which was damaged, stored in warm conditions or not completely dry. Elevated Peroxide and Anisidine Values can in many cases be related to oxidative damage to the oil during processing where high temperature, exposure to oxygen or pro-oxidant metals could be identified

Interpretation of hydrolytic and oxidative deterioration

Although hydrolytic deterioration and oxidative deterioration normally correspond with the level of degradation, the contrary is often true. With Marula oil for example, free fatty acid formation is a problem due to the difficulty of drying the Marula seed covering surrounding the kernel. After the fruit is removed from the Marula seed, it is important to get the seeds as clean as possible from remaining sugars and moisture, which can become a substrate for the growth of micro-organisms. These micro-organisms secrete enzymes which can cause hydrolytic deterioration of the oil by removing the fatty acids from the glycerol backbone. This can be prevented if the seed is clean and dry. Drying needs to be exhaustive, whether by sun, air or microwave.

Although there are certain rules which apply to ensuring the oxidative stability of oils, there are some oils that behave differently under certain processing conditions. Marula oil, for example, has high concentrations of natural antioxidants which protect the oil against oxidative deterioration, and high levels of free fatty acids are often accompanied by extremely low PVs.

The best advice is to monitor the production process of each oil, from the moment the fruit is collected to the final storage of the oil, while keeping in mind the factors causing hydrolytic and oxidative deterioration. The extraction and storage process contributes to maintaining the quality of the oil.

General compositional tests for fats and oils

Saponification Value (SV)

Saponification literally means 'soap making'. The SV is an indication of the suitability of an oil for soap making. It is a reflection of the chain length of the fatty acids present in the fat or oil, and is a measure of the average molecular weight of all the fatty acids present.

The higher the SV, the shorter the fatty acids and vice versa (*Table 1*). The shorter the fatty acids, the better the oil for soap making.

The SV is expressed as the number of milligrams of potassium hydroxide (KOH) required to saponify one gram of the test sample. It is applicable to all normal fats and oils. Therefore, the shorter the fatty acids in a specific amount of oil, the more alkali nescessary to release them from the glycerol back bone.

Table 1. *Typical Saponification Values indicating their inverse relationship with chain length*

Type of oil	Saponification Value	Main fatty acid
Coconut oil	248-265	C12 (45-83%)
Sunflower seed oil	188-198	C18:2 (48-74%)
Baobab seed oil	190-198	C18:1 (20-40%)
Kalahari melon seed oil	180-200	C18:2 (60-70%)

Unsaponifiable matter

Unsaponifiable matter includes substances frequently found dissolved in fats and oils (*Figure 8*), which cannot be converted to soap by the usual caustic treatment, but are soluble in ordinary fat and oil solvents. Included in this group of compounds are higher aliphatic alcohols, sterols, pigments and hydrocarbons.

The level of unsaponifiables in oil is an indication of whether the oil has been refined or not. Refined oil has an unsaponifiable content below 1%. Most crude oils contain unsaponifiables varying between 1% and 2% (*Table 2*). Crude Shea butter is an exception with levels exceeding 7%. The unsaponifiable matter is expressed as g/100g oil.

Hemp (*Cannabis sativa* L.) seed oil: Analytical and phytochemical characterization of unsaponifiable fraction

Figure 8. *Different components in oil*

Oil type	Unsaponifiable matter content (g/100g oil)
Crude soybean	< 1.5
Refined soybean	< 1.0
Crude Shea butter	7 – 17
Refined Shea butter	Min 4

Table 1. *Typical unsaponifiable matter content of various oils*

Iodine Value (IV)

The IV Indicates the degree of unsaturation of a fat or oil. It is defined as the number of grams of iodine absorbed by 100g of fat or oil. The higher the IV, the more double bonds present in the fat or oil. Coconut oil is a saturated fat and will have a low IV, while grapeseed oil is a mostly polyunsaturated fat with a high IV. There are two types of IV determinations. One is done according to the Wijs method which entails wet chemistry. The other method is a theoretical determination based on the fatty acid profile determined by gas chromatograph. Both methods are equally accurate if all fatty acids can be quantified by the gas chromatograph method. Typical IVs are depicted in Table 2.

Oil type	Iodine Value
Coconut oil	6 - 10
Marula oil	70 - 80
Sunflower oil	118 - 141
Grapeseed oil	128 - 150
Marula Oil	120 - 140

Table 2. *Typical Iodine Values of vegetable oils*

Relative density

Relative density, or specific gravity, is the ratio of the density of a substance to the density of a reference material. Oil is measured against water at a certain temperature, usually 20°C. This is done by comparing the mass of oil in a specific volume to the mass of water in the same volume. The density of oil is always lower than that of water and the ratio will therefore always be less than one. The density of vegetable oils generally ranges between 0.890 and 0.920.

Refractive Index

Refractive Index measures the bending of light when passing from one medium to another. It is an indication of purity as well as unsaturation of the oil. The Refractive Index of vegetable oils usually ranges between 1.44 and 1.47.

Authenticity testing of oil

Ensuring the authenticity of food has been a problem for many years. Wherever there is a product with a high value, such as Marula or Baobab oil, people are tempted to profit by diluting or adulterating it with a lower value oil. Food fraud usually involves misleading the purchaser as to the true nature or origin of the product.

The vegetable oil sector has products of differing sales volumes and values. Speciality oils are extracted from seeds which are scarce or harvested ethically or sustainably, and high prices are paid for these oils. Diluting a speciality oil with a commodity oil means it can be sold at a lower price than that of competitors. This causes market prices to become distorted and makes it impossible for ethical producers to compete.

The international oil industry has set standards to curb the sale of adulterated oil. These tests include the fatty acid profile, tocopherol and sterol analysis. Oils considered as commodities are evaluated by the Codex Commission and the ranges for the tests are published in the *Codex Alimentarius*. It is important to create these standards for speciality indigenous oils which are produced from scarce plant material and are very costly. Adulteration of these oils with lower priced commodity oils is a realistic threat to the industry. Creating standards based on the fatty acid profile as well as the tocopherol and sterol content will ensure that authenticity is verified prior to each sale and adulteration and distortion of market prices is prevented.

Fatty acid profile

The fatty acid profile of a fat or oil is like a human fingerprint and helps to differentiate between different vegetable oils. Each type of oil has a unique fatty acid profile with a typical range. These ranges are usually determined by seasonal, geographical and other environmental factors over a wide range of samples. The fatty acid profile of oil can give a lot of information, including the expected shelf life, physical properties and functionality in a cosmetic or food product.

The fatty acid profile is one of the most important tools to verify the authenticity of an oil. If the fatty acid profiles of two blended oils are very different, the adulteration can be identified based on the fatty acid profile alone.

Fatty acid profiles of oils can, however, be very similar, and when oils are blended cleverly it is nearly impossible to detect the adulteration. It is therefore important to also test oils for their micro-components. According to the *Codex Alimentarius*, the two most important groups to test are the sterols and tocopherols. If the fatty acid profile, tocopherol and sterols have been identified and quantified and reported for a sample, it is nearly impossible to release an adulterated sample into the marketplace.

Tocopherols

Tocopherols are a family of organic compounds that have Vitamin E activity. ***Vitamin E*** exists in eight different forms and is a general term employed for the four tocopherols and four ***tocotrienols***, including α (alpha), β (beta), γ (gamma), and δ (delta) species. These eight compounds have different biological activity and antioxidant capacity. The main food sources are vegetable oils, nuts, ***oil seeds***, ***wheat germ*** and food products prepared with any of these ingredients (such as margarine).

The most active form of this vitamin is α tocopherol, which is widely distributed in nature and is often used in vitamin supplements. Although natural Vitamin E is generally alpha tocopherol, it can sometimes appear as natural mixtures of beta, gamma, and delta tocopherols. Tocopherols contribute to the antioxidant properties of oil, and its profile and composition are often criteria of purity. The importance of these compounds is supported by verifiable evidence of their role in the quality and authenticity of oil as well as their contribution to human health.

Every type of oil has a unique combination of tocopherols and tocotrienols. This profile is used in combination with the fatty acid profile and sterol composition to verify authenticity of oil.

Sterols
Plant sterols are a group of substances made in plants. Plant sterols are found in the highest amounts in foods like vegetable oils, nuts, and seeds.

Plant sterols have medicinal purposes and are most commonly used for lowering cholesterol. Sterols can be present in the free form and as fatty acid esters and glycolipids. Although sterols do not seem to have a stabilising effect of oil, they are said to help block the body from absorbing cholesterol. While plant sterols help lower LDL cholesterol, they don't appear to affect levels of HDL cholesterol or triglycerides .

Vegetable oils and products have been identified as sources of plant sterols, highlighting phytosterol content. Cereal products, vegetables, fruit and berries, which are not as rich in phytosterols, may also be significant sources of phytosterols due to their higher intakes. Along with the profile of fatty acids, triglycerides and tocopherols, the sterol profile is an important parameter to assess the identity and authenticity of fats and oils. Sterols are removed during the deodorisation step of ***refining oils*** and fats without changing their relative composition, and are therefore a useful tool in checking authenticity.

Refining of oils

Crude oils obtained by oilseed processing often have to be refined before consumption or use in cosmetic ingredients to remove any undesirable accompanying substances.

There are two types of refining: physical refining and chemical refining. The names physical and chemical refining come from the technology used to remove the free fatty acids (FFA) that are responsible for the oil acidity. Chemical refining uses chemicals to remove the FFA, while physical refining uses steam. The choice of which process to use depends on the types and qualities of the crude oil to be processed. The rest of the steps are similar and include degumming, dewaxing/winterisation, bleaching and deodorisation (*Figure 9*).

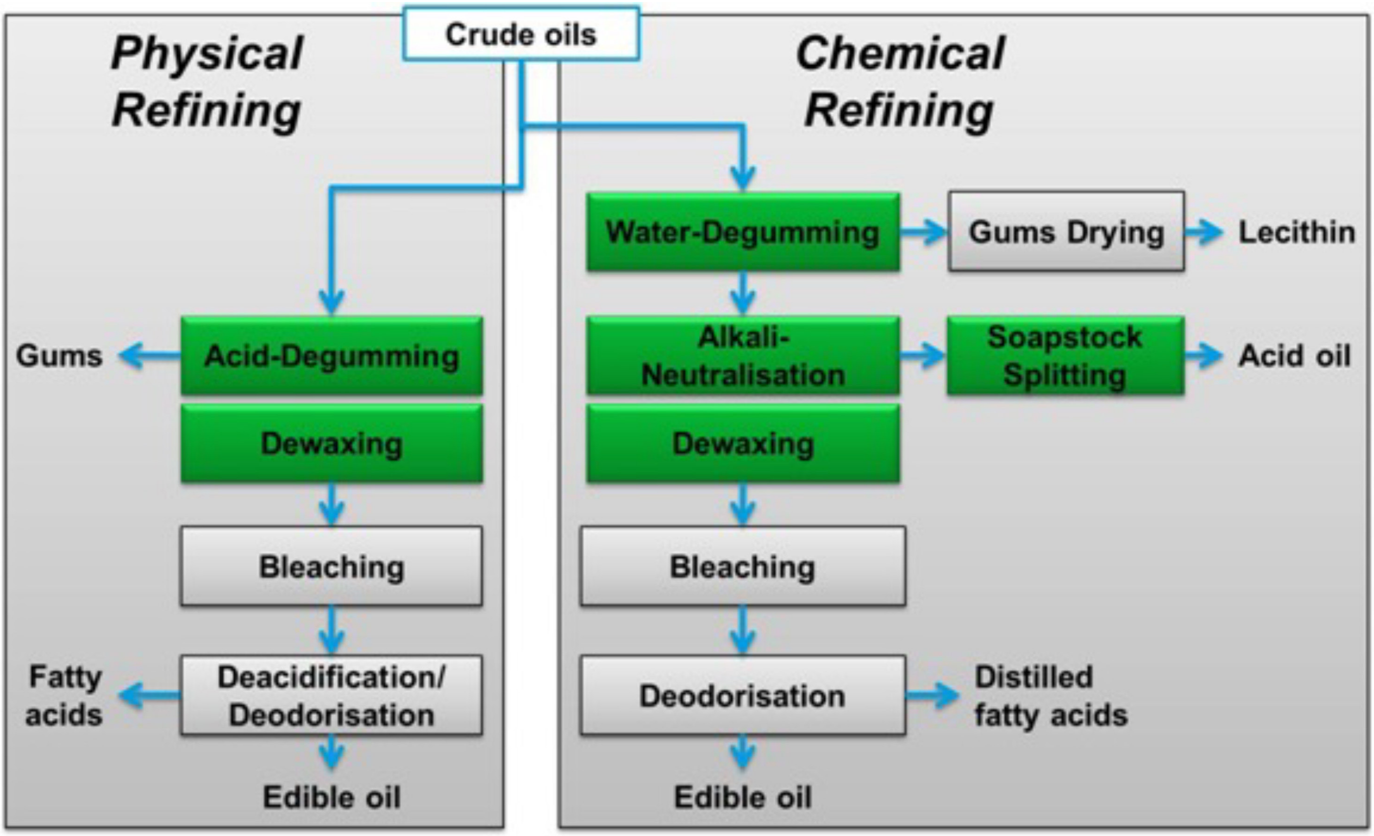


Figure 9. *Physical and chemical refining of vegetable oil*

The traditional alkali refining is often replaced by physical refining in which the use of chemicals is reduced. The most widely-used physical refining method is steam refining. The crude oil quality is very important in order to obtain high quality refined oil. A low-quality crude oil cannot result in a high-quality refined oil. The oil might appear to be of high quality, but structural damage to the crude oil due to hydrolytic and oxidative deterioration prior to refining will remain and reduce the shelf life of the refined oil.

The advantage of a refined oil is that the FFA content and Peroxide Value is lower and the oil has a neutral odour and light colour. This makes it ideal for application in the food and cosmetic industry, where low odour and light colour is required. The disadvantage of refined oils is that natural antioxidants are partially removed during refining and the typical colour and odour of the oil is lost. It is very important that crude oil is always produced in a way that the quality of the oil is preserved. This is not only important when selling oil to a market requiring crude oil, but also to ensure prolonged shelf life of oil that is intended for refining.

Chemical refining

Chemical refining involves degumming, alkali neutralisation, dewaxing/winterisation, bleaching and deodorisation (*Figure 9*).

Degumming

Degumming is the process of hydrating phosphatides in an oil by adding water, followed by centrifugation. There are three reasons to degum oil: to produce lecithin (phosphatides), to provide degummed oil for long-term storage or transport, and to prepare it for refining.

Neutralisation

Neutralisation is the process of removing FFAs from crude oils by using caustic soda or lime. After addition of alkaline solutions, soap is formed in the oil and removed by washing with hot water; and excess water is removed before further processing.

Winterising

Most crude vegetable oils contain non-triglyceride compounds that create a haze or wax in the oil when chilled or stored for long periods. Chilling, followed by filtration, removes these compounds. This process in oil refining, where high-melting components are removed, is known as winterising or dewaxing.

Bleaching

The bleaching step is performed to remove colour pigments from vegetable oils. The neutralised and winterised oil is heated through thermic boilers to raise the temperature to 130°C. The oil is then treated with bleaching clays that adsorb the colour pigments.

Deodorisation

Deodorisation is the last step of refining and uses a steam-distillation process to remove FFAs and volatile components in edible crude oil. These unwanted substances negatively affect the odour, flavour, colour and stability of the final product. Unfortunately this step also removes some desired compounds from oil, including tocopherols and sterols. After the deodorisation step, refining is complete. The major differences between crude and refined vegetable oils are listed in table 3.

Table 3. *Major differences between crude and refined oil*

Crude oil	Refined oil
Higher FFA	Lower FFA
Higher PV	Lower PV
Darker colour	Lighter colour
Contains natural antioxidants	Stripped of most natural antioxidants
Contains more waxes	Contains less waxes

Physical refining

The major difference between chemical and physical refining is the process where FFAs are removed. Where chemical refining uses FFAs, physical refining uses steam. This step consists of the application of superheated steam under low pressure and at temperatures higher than 220°C. FFAs as well as objectionable volatiles, formed by the cleavage of lipid oxidation products, are removed.

A disadvantage is the partial loss of tocopherols and sterols. Side reactions, particularly isomerisation of polyunsaturated fatty acids, should be minimised. The quality of physically refined oil is close to that of alkali refined oils, but losses of neutral oil are lower and the environment is less polluted with chemical waste. The use of selective membranes for physical refining is promising.

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