

# Chapter 2

## PRODUCTION OF LIPIDS FROM NATURAL SOURCES

*More than 80 million tones of oils and fats are available annually from various natural sources, including plants, animals and fish etc. This chapter lists major sources of lipid production and explains their properties.*

### 2-1 Plant Oil

There are two types of oils in plants. The first one is oil in terms of energy storage in plants, which usually reserved in seeds. The second one is plant essential oil which are produced as self-defense or attractant compounds. Most of plant oils are used in several industries such as food, beverage, painting, and perfumery. Methods of extraction plant oils can be done in several ways.

#### 2-1-1 Extraction methods

**Steam distillation:** Plant material is placed into a still (very similar to a pressure cooker) where pressurized steam passes through the plant material. The heat from the steam causes globules of oil in the plant to burst and the oil then evaporates. The oil vapor and the steam then pass out through the top of the still into a water-cooled pipe where the vapors are condensed back to liquids. At this point, the essential oil separates from the water, for certain oil, if the density is greater than 1, it will fall down to the bottom, however, most of the oil float on top.

**Maceration:** This method, plant material is soaked in vegetable oil, so, oil from plant will infuse to vegetable oil, this method can be used for certain species of plants.

**Enfleurage:** This technique is similar to maceration but different technique is used. Instead of soaking plant material in vegetable oil, plant material is applied on top of animal fats, e.g. lard. Plant oil will infuse into fats and later on, fats will be dissolved in solvent like hexane or ethanol, plant oil separates from fats.

**Cold pressing:** Cold pressing is used to extract the oils from citrus rinds such as orange, lemon, grapefruit and bergamot. The rinds are separated from the fruit, are ground or chopped and then pressed. The result is a watery mixture of oil and liquid which will separate in given time. Oil extraction using this method has a short shelf-life.

**Solvent extraction:** Organic solvent is added to plant material to help dissolve oils. When the solution is filtered and concentrated by distillation, a substance containing resin (resinoid), or a combination of wax and oil (known as concrete) remains. From the concentrate, pure alcohol is used to extract the oil. When the alcohol evaporates, the oil is left behind.

**Carbon dioxide extraction (Supercritical carbon dioxide extraction):** This method is recently developed. Carbon dioxide is used to extract oil from plant when liquefied under high pressure. Plant materials are placed in a stainless steel tank. Once the liquid depressurizes, carbon dioxide returns to a gaseous state, and only pure oil remains.

## 2-1-2 Plant oils

Vegetable oils have been used in daily lives since ancient time. Sesame oil is considered to be the oldest oilseed crop, later on, some other crops such as soybean, canola, corn and sunflower have been exploited in human lives. Plant oils are complex mixtures of natural substances. Most of plant oils are extracted from seed, storage structure of origin of plant life. Plant produces fatty acid, protein and/or carbohydrate and stores them in seed as reserved energy source during germination. After seed imbibition, the stored energy source are broken down and then utilized by embryo. When embryo is well developed, it can produce its own energy through photosynthesis. Plant oil properties are different from those of animal. Most of the plant oils contain unsaturated fats whereas animal oil is highly in saturated fats. Even though human can convert carbohydrates into fats, there are some types of fatty acids that need to be incorporated with food, such as linoleic acid and linolenic acid which can be found in plant oils. Types of fatty acids found in vegetable oils are illustrated in Table 2-1-1.

Normally, plant oils have high content of monounsaturated and polyunsaturated fatty acids. Only oils from palm kernel and coconut that have great amount of saturated fatty acid especially for 16:0, which is similar to the fatty acid found in animal fats.

## 2-1-3 Plant essential oils

Essential oils can be extracted from different plant parts such as flower, fruit, leaf and wood. Nowadays, essential oils have been used widely in perfumery, food flavoring and cosmetic industries. Major components of essential oils are terpenes, fatty acid derivatives, amines and benzene derivatives.

**Products of terpene biosynthesis:** Terpenes, major components of plant essential oils, are derived from mevalonic acid pathway (Figure 1-9-2). Mevalonic acid is converted to geranylpyrophosphate, the most important precursor of monoterpene biosynthesis pathway. Then geranylpyrophosphate is further modified to form various monoterpenes and other larger terpenoids.

Some examples of plant essential oils are listed in Table 2-1-2.

Seeds	Fat Content (%)	Fatty acid composition (%)				
		Polyunsaturated		Monoun-saturated	Saturated	
		Linolenic acid (18:3 $\omega$ -3)	Linoleic acid (18:2 $\omega$ -6)	Oleic acid (18:1 $\omega$ -9)	Stearic acid (18:0)	Palmitic acid (16:0)
hemp	35	20	60*	12	2	6
chia	30	30	40	-	-	-
kukui	30	29	40	-	-	-
flax	35	58	14	19	4	5
pumpkin	46.7	0-15	42-57	34	0	9
soybean	17.7	7	50	26	6	9
walnut	60	5	51	28	5	11
wheat germ	10.9	5	50	25	18	0
evening primrose	17	-	81**	11	2	6
safflower	59.5	-	75	13	12	-
sunflower	47.3	-	65	23	12	-
grape	20	-	71	17	12	-
corn	4	-	59	24	17	-
sesame	49.1	-	45	42	13	-
rice bran	10	1	35	48	17	-
cottonseed	40	-	50	21	25	-
canola	30	7	30	54***	7	-
peanut	47.5	-	29	47	18	-
almond	54.2	-	17	78	5	-
olive	20	-	8	75	16	-
avocado	12	-	10	70	20	-
coconut	35.3	-	3	6	0	91
palm kernel	35.3	-	2	13	0	85
beech	50	-	32	54	8	-
brazilnut	66.9	-	24	48	24	-
pecan	71.2	-	20	63	7	-
pistachio	53.7	-	19	65	9	-
hickory	68.7	-	17	68	9	-
filbert	62.4	-	16	54	5	-
macadamia	71.6	-	10	71	12	-
cashew	41.7	-	6	70	18	-
neem	40	1	20	41	20	-

\*Includes up to 2%  $\gamma$ -linolenic acid (GLA).

\*\*Includes 9% GLA. \*\*\*Includes up to 5% erucic acid.

Table 2-1-1: Fats content and fatty acid composition of some seed oils.

Plant name	Chemical composition
Allspice	eugenol, methyl eugenol, cineol phellandrene, caryophyllene
Aniseeds	anisaldehyde, anethole, methylchavicol, limonene
Basil	linalool, camphor, pinene, eugenol, methylchavicol
Bay leaf	eugenol, chavicol, myrcene, cineol, methyleugenol
Bergamot	limonene, linalyl acetate, linalool, $\gamma$ -terpinene, bergaptene
Black pepper	eugenol, safrole, thujane, farnesene, $\alpha$ -pinene, $\beta$ -pinene, sabinene, limonene, $\beta$ -caryophyllene, camphene
Chamomile	angelic acid, methacrylic acid, butyric acid, tiglic acid, azulene
Camphor	cineol, camphor, borneol, camphene, menthol, $\alpha$ -pinene, $\beta$ -pinene, <i>d</i> -limonene
Cinnamon	eugenol, cinnamaldehyde, $\beta$ -caryophyllene, linalool, methylchavicol
Cloves	eugenol, furfural, caryophyllene, eugenylacetate, $\alpha$ -pinene, $\beta$ -pinene
Eucalyptus	camphene, citronellal, fenchone, phellandrene, cineol
Geranium	geraniol, citronellol, citronellyl formate, linalool, myrtenol, terpineol, citral, menthone, sabinene
Ginger	borneol, citral, cineol, zingiberene, camphene, limonene, phellandrene, $\beta$ -bisabolene
Jasmine	nerol, terpineol, linalyl acetate, methyl anthranilate, jasmone, farnesol
Lavender	borneol, geraniol, linalool, lavandulylacetate, linalyl acetate, cineol
Lemon	<i>d</i> -limonene, citronellal, phellandrene, citral, citrioptene
Lemongrass	citral, farnesol, nerol, citronellal, myrcene
Neroli	$\alpha$ -pinene, limonene, linalyl acetate, linalool, nerolidol, nerol, geraniol, citral
Orange	<i>d</i> -limonene, linalool, terpineol, $\beta$ -carotene
Peppermint	menthol, menthyl acetate, carvone, menthone, carvacrol, <i>d</i> -limonene
Rose	citronellol, geraniol, nerol, farnesol, eugenol
Rosewood	geraniol, linalool, nerol, cineol, terpineol, <i>d</i> -limonene
Sandalwood	santalol, furfural, santalene
Thymes	linalool, carvacrol, thymol, borneol, caryophyllene, terpinene
Tea tree	terpinene-4-ol, cymene, $\alpha$ -pinene, terpinene, cineol
Ylang ylang	geraniol, linalool, eugenol, safrole, farnesol, $\alpha$ -pinene, $\beta$ -pinene

Table 2-1-2: Common chemical composition of plant essential oils.

## 2-2 Animal Oil (Animal Fat)

	% total fats			
	SFA	MUFA	PUFA	Others
Beef, lean, cooked	38.2	42.1	3.4	16.3
Pork, lean, cooked	35.4	44.7	7.5	12.4
Lamb, lean, cooked	35.7	43.8	6.5	14.0
Veal, lean, cooked	28.0	35.7	9.0	27.3
Chicken, cooked	27.5	35.9	22.8	13.8
Tuna, canned in water	28.5	19.4	41.1	11.0
Salmon, cooked	16.2	27.1	39.2	17.5
Butter	62.2	28.8	3.7	5.3
Corn oil	12.7	24.2	58.7	4.4
Olive oil	13.5	73.7	8.4	4.4

SFA = saturated fatty acid,  
 MUFA = monounsaturated fatty acid,  
 PUFA = polyunsaturated fatty acid.

Table 2-2-1: Relative proportion of types of fatty acid and other lipid, found in animal meat vs. vegetable oils.

Animal oil property is different from vegetable oil in terms of type of fatty acid. Most of animal oil fatty acids are saturated ones. Comparison between animal and vegetable oil reveals that animal oils contain saturated fatty acid greater than that of vegetable one (Table 2-2-1).

Major fatty acids in meat are myristic (14:0), palmitic (16:0), stearic (18:0), palmitoleic (16:1  $\omega$ -9), oleic (18:1  $\omega$ -9), linoleic (18:2  $\omega$ -6), linolenic (18:3  $\omega$ -3) and arachidonic (20:4  $\omega$ -6). In addition, other product from animal, for example milk, has various type of fatty acid (Table 2-2-2).

Animal fats can be obtained in the course of dressing carcasses of slaughtered animals or at a later stage in butchering process when meat is being prepared for final consumption. Lard, oil from pig, can be obtained by melting raw pig fats and tallow, oil from other animal (other than pigs), can be prepared from raw fat in a similar method of pig one. Animal fats are used for margarine and shortening production. Some can be used in soap production, fatty acids, lubricants and feeds industries.

Generally speaking, animal oils are normally solidified at room temperature whereas vegetable oils are in liquid form. Thus, sometimes, words like "animal fats" are used in stead of "animal oils".

4:0	6:0	8:0	10:0	12:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2
3.32	2.35	1.19	2.80	3.39	11.39	2.62	29.46	3.37	9.87	27.45	2.80

Table 2-2-2: Types of fatty acid found in milk (%).

## 2-3 Genetically modified crops for oil seeds

Vegetable oils are in high demand in everyday life. They are used in cooking, making margarine and other processed foods and also in producing several non-food items such as soap, cosmetics, medicine and paint. Vegetable oils tend to contain more unsaturated fatty acids than animal fats. They are usually in liquid form at room temperature. Plants produce fatty acids with a wide variety of chain lengths and degrees of saturation. Each combination gives different properties and, therefore, different functions of oil.

There are several different sources of vegetable oils, for example, soybean, canola, palm seed, sunflower, corn and castor. Oils can be extracted from seeds. The advantage of vegetable is cholesterol-free and low in saturated fat.

Plant breeders have been looking for ways to improve these crops in order to obtain high quality of oil. However, with conventional methods, it will take a long process to produce a desirable cultivar. New technique, genetic engineering method, offers way to produce new plant cultivar in a short time. There are quite a number of researchers that have plans to alter genes of oil plant to improve oil quality and quantity. There are two crops, canola and soybean, that genetic engineering has been exploited and new genetically engineered cultivars can be released for use.

### 2-3-1 Improvement of oil content in rapeseed (canola)

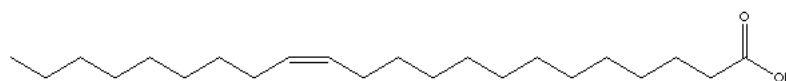


Figure 2-3-1: Structure of erucic acid.

Although not “genetically modified”, canola is a very important example for improved oil seed crops. Rapeseed (*Brassica napus*) oil naturally contains large amounts of erucic acid (22:1 ω-9, Figure 2-3-1). It used to be in high demand as lubricant. Since erucic acid is not safe for human consumption, and demand of vegetable oil has been increased, breeders have been working to reduce the amount of erucic acid and found new rapeseed that can be used as cooking oil. Rapeseed that has low content of erucic acid is called canola.

### 2-3-2 High erucic acid

Line	Oil content (% of DW)	Erucic acid (%wt/wt)	Seed yield (g/plot)
Control	41.5 ± 1.5	46.5 ± 1.5	657 ± 97.8
H-5-1-4	46.0 ± 1.2	52.1 ± 1.5	875.5 ± 88.5
H-5-4-8	47.4 ± 0.5	51.6 ± 0.5	1478 ± 86.4
H-8-6-8	44.3 ± 0.4	48.9 ± 0.4	795.7 ± 35.6
H-8-7-2	44.9 ± 1.0	50.7 ± 1.0	832.3 ± 14.5
H-8-10-2A	46.1 ± 0.8	53.4 ± 0.9	881.5 ± 245.5
H-8-10-5	46.0 ± 0.3	51.0 ± 0.6	827 ± 73

Table 2-3-1: Comparison of regular rapeseed and transgenic rapeseed in terms of erucic acid content, oil content and seed yield.

Rapeseed oil which has high amount of erucic acid is in great demand for plastic film manufacture, synthesis of nylon, lubricant and emollient industries as well as photographic materials.

Researchers from Plant Biotechnology Institute, Canada, transformed rapeseed plant by adding lysophosphatidic acid acyltransferase (LPAT) gene (SLC1-1 gene) from baker's yeast (*Saccharomyces cerevisiae*). LPAT is an enzyme that is involved in triacylglycerol biosynthesis. The LPAT from yeast is able to incorporate erucic acid into glycerol backbone while the one from rapeseed does not accept it very much. The incorporated yeast LPAT gene showed some effects on increasing amount of erucic acid in rapeseed as well as oil content and average seed weight (Table 2-3-1).

Even though the amount of erucic acid is not as great as expected, the transgenic already showed some promising potential in obtaining a cultivar that can produce high erucic acid. Further test and selection are underway.

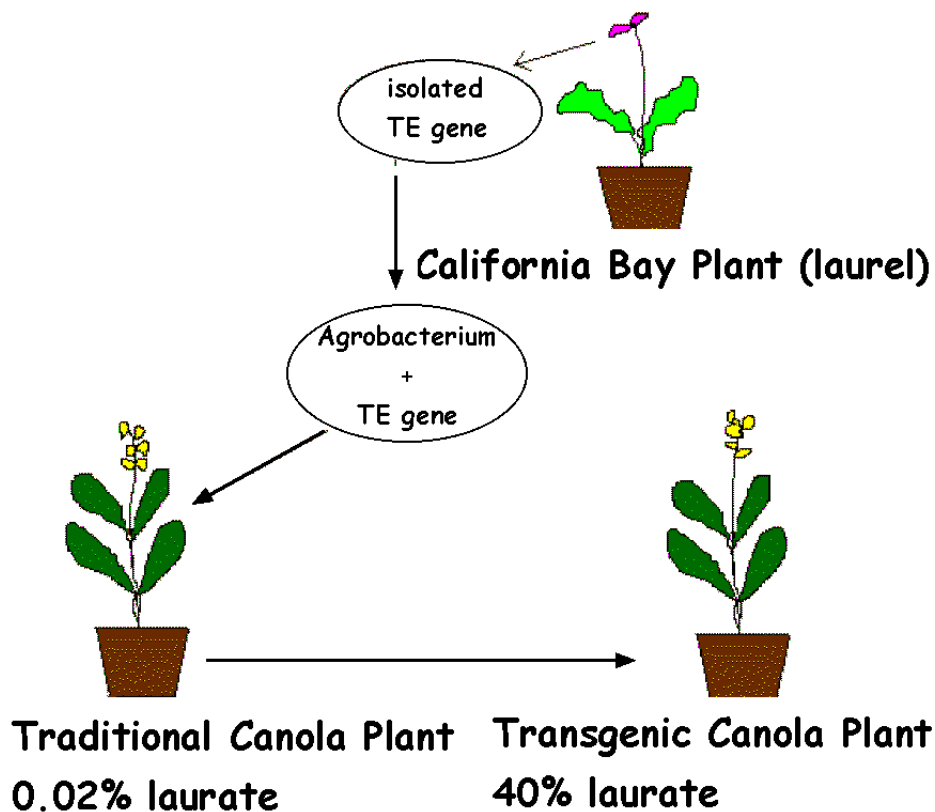


Figure 2-3-2: Illustrate how transgenic canola is derived.

### 2-3-3 High lauric acid

Until recently, with the new technique of genetic engineering, canola has been changed in its oil content in terms of lauric acid from 0.02% to about 40%, which is in high demand in soap and detergent manufactures.

Rapeseed (canola) has been genetically engineered to modify its oil content by Calgene (now Monsanto). A gene encoding 12:0 acyl carrier protein thioesterase (12:0 TE)

from California bay plant (*Umbellularia carifornica*, laurel tree) was added into canola, via *Agrobacterium tumefaciens* in order to increase the level of medium chain length fatty acids, in particular lauric acid.

In most ordinary plants, the predominant thioesterases remove growing acyl chains of 16 or 18 carbons in length, during fatty acid biosynthesis. The 12:0 TE removes, however, growing acyl thioester chains from the acyl carrier protein (ACP) once they get to be 12 carbons long. Therefore, expression of the 12:0 TE gene from bay plant in canola causes “premature” hydrolysis of the 12:0 acyl thioester chains, resulting in accumulation of lauric acid.

Because the 12:0 TE gene is linked to seed-specific promoter, it is turned on only in the seeds of the plant, from which oil is extracted. Products from this genetically engineered variety has to be labeled as “high laurate canola”. Figure 2-3-2 illustrates how transgenic canola is derived.

### 2-3-4 High oleic acid soybean



Figure 2-3-3:  $\Delta$ -12 desaturase converts oleic acid to linoleic acid.

Soybean has been used as a good source for polyunsaturated fatty acids. However, these fatty acids are very sensitive to rancidity, making it unsuitable for many food uses. Most of the manufacturers have to hydrogenate soybean oil to increase its stability, nevertheless, this also increases levels of trans-fatty acids, which are associated with atherosclerosis and other nutrition-related chronic diseases.

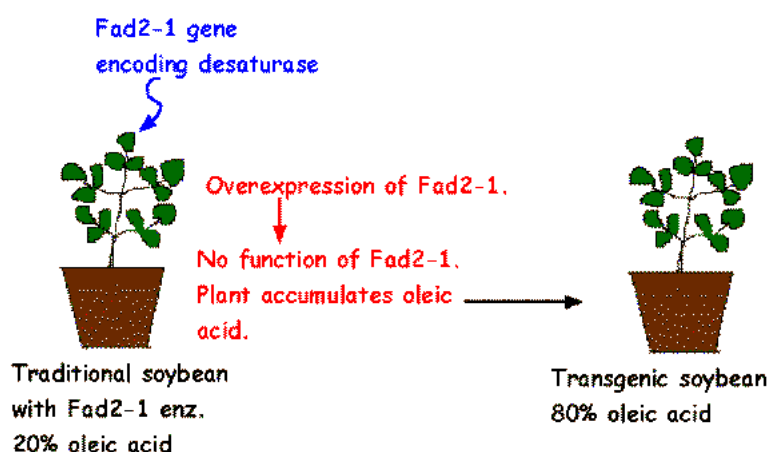


Figure 2-3-4: Co-suppression of *Fad2-1* gene on soybean plant.

An attempt was made to reduce the content of polyunsaturated fatty acid in soybean oil (mainly linoleic acid, 18:2  $\omega$ -6). In developing soybeans, the second double bond is



added to oleic acid in the  $\Delta$ -12 ( $\omega$ -6) position by  $\Delta$ -12 desaturase, encoded by *Fad 2-1* gene (Figure 2-3-3). “Co-suppression technique” has been employed by DuPont to reduce levels of the polyunsaturated fatty acids using *Fad2-1* gene. When double amount of the *Fad2-1* gene occurs inside the plant, plant can detect the overexpression of it thus, these genes, original version and the genetically engineered one, are turned off (this phenomenon is called co-suppression). Therefore, plant produces less unstable unpolysaturated fatty acid and accumulates oleic acid, from 20% to 80% (Figure 2-3-4). Oil of the new genetically engineered variety is stable, does not require chemical hydrogenation and does not produce *trans*-fatty acid byproducts of hydrogenation.

## 2-4 Fish Oil

One of the most important characteristic features of fish oil is their high content of  $\omega$ -3 polyunsaturated fatty acids ( $\omega$ -3 PUFA) (Table 2-4-1). The  $\omega$ -3 PUFA have attracted great interest for their nutritional and pharmaceutical applications, since Dyerberg and his co-worker’s comparative study between Greenland Eskimos and the Danish people revealed that oils from fish and sea animals had anti-blood coagulation effects. One of the major fish oil PUFAs, eicosapentaenoic acid (EPA, 20:5  $\omega$ -3) is known to have anti-coagulation and anti-atherosclerosis effects, while another one, docosahexaenoic acid (DHA, 22:6  $\omega$ -3) likely involved in brain health such as memory-improvement.

Fish	Fatty acid composition (%)										
	14:0	16:0	16:1	18:0	18:1 $\omega$ -9	18:2 $\omega$ -6	18:4	20:1	20:5 $\omega$ -3	22:1	22:6 $\omega$ -3
Sardine	8	20	8	3	13	3	4	5	13	4	11
Mackerel	4	19	5	5	27	1	2	4	9	3	13
Horse mackerel	4	23	8	7	23	1	1	1	8	1	15
Pacific saury	8	11	4	2	7	2	4	17	6	20	11
Flatfish	4	17	11	4	16	-	1	3	15	1	14
Herring	8	14	7	1	22	1	2	12	7	14	6
Bonito	3	24	4	10	16	1	1	1	6	1	25
Tuna	3	20	4	9	25	1	1	5	4	5	16
Flying fish	3	21	4	8	10	1	1	1	6	-	35
Squid	2	26	-	6	4	-	-	3	14	-	39
Cod liver	4	11	8	2	22	1	3	12	11	23	8
Shark liver	3	13	10	3	23	1	1	8	12	5	13

Table 2-4-1: Fatty acid composition of major fish oil.

Fish oils containing 30% of  $\omega$ -3 PUFA, which are combined in mixed triacylglycerols with ordinary fatty acids, are the main source of  $\omega$ -3 PUFA. These PUFA-containing oil can be used as food additives or supplements.

Because each PUFA species has its unique biological activity, it is often necessary to use a highly purified particular PUFA species. Molecular distillation is one of the versatile industrial methods for purification of fatty acid species. However, PUFA species of fish oil cannot be purified directly by molecular distillation because of their relatively low stability. Their multiple double bonds are easily oxidized and polymerized at high temperatures. Therefore, fish oils are transformed into ethyl or methyl esters, which have lower boiling points and can be more easily separated by vacuum distillation. The  $\omega$ -3 PUFA ethyl or

methyl esters are the most common commercially available forms of high purity PUFA. The highly purified EPA-ethyl ester is marketed as a medicine for cardiovascular diseases.

## 2-5 Single cell oil

Single cell oil (SCO), a new category of oil source, is defined as oils produced by microorganisms (= single cell organisms). The use of SCO is limited to the production of oils containing particular fatty acids, of which appropriate industrial sources are not available by conventional means (plants, animals and fish). For this reason, the researches of SCO production have been focused on certain kinds of polyunsaturated fatty acids with special values and functions. A typical production process of SCO is summarized in Figure 2-5-1. The oil-producing strain is cultivated, then the cells are recovered and dried. From the dried cells, oils is extracted solvent extraction.

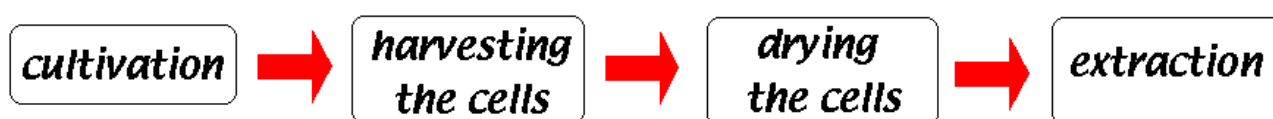


Figure 2-5-1: Production of SCO.

### 2-5-1 $\gamma$ -Linolenic acid (GLA, 18:3 $\omega$ -6)

$\gamma$ -Linolenic acid (GLA) is an  $\omega$ -6 series fatty acid with special biological function such as anti-allergy, anti-inflammatory and improvement of lipid metabolism etc. However, natural source of GLA was very limited; it was found only in some special plants such as evening primrose. In 1985, Dr Suzuki and coworkers isolated a fungal strain, *Mortierella* sp. which accumulated GLA in the cells. After their discovery, fermentational production of GLA using this fungal strain was industrialized. This was the first example of industrial SCO production.

### 2-5-2 Arachidonic acid (ARA, 20:4 $\omega$ -6) and dihomo- $\gamma$ -linolenic acid (DHGLA, 20:3 $\omega$ -6)

A research group led by Profs. Shimizu and Yamada in Kyoto University found a new fungal strain, *Mortierella alpina*1S-4 as a high ARA- producing strain. The finding of the fungal strain enabled the industrial production of ARA by fermentation. ARA-containing oil can be obtained from the dried mycelium (= the cell of the fungus) by solvent extraction similarly to the case of plant oil. Nowadays, ARA-containing oil is produced industrially.

In addition, they isolated a mutant of the 1S-4 strain having lower  $\Delta$ -5 desaturase activity than the parent. Using this mutant strain, the research group achieved dihomo- $\gamma$ -linolenic acid production, which had never been possible until that.

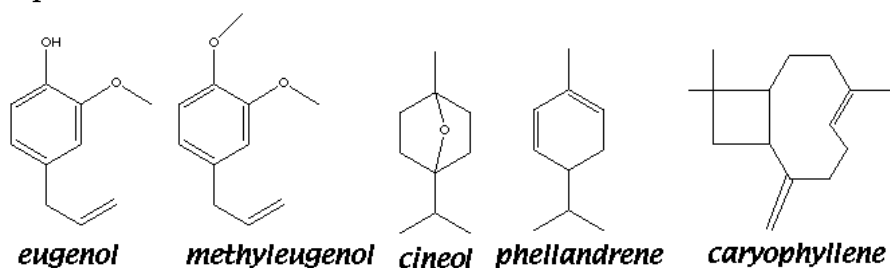
### 2-5-3 Eicosapentaenoic acid (EPA, 20:5 $\omega$ -3) and docosahexaenoic acid (DHA, 22:6 $\omega$ -3)

It seems that SCO containing EPA and DHA are not industrially utilized, because there is already a well-established industrial source for EPA and DHA, i.e. fish oil. In fact, microorganisms producing EPA and DHA were identified, and the industrial scale fermentation

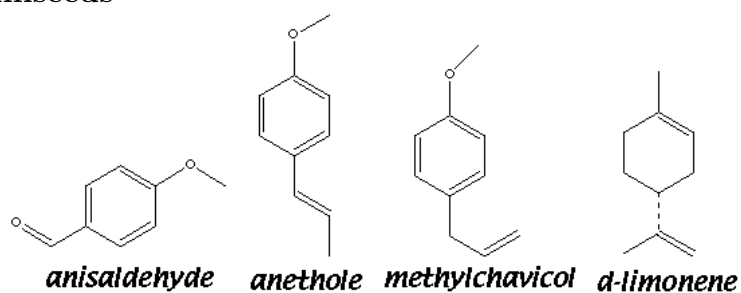
of such SCO are in progress. For instance, *Schizochytrium* sp. SR21 strain produces oil containing 30-50% of DHA. Since this oil contains only DHA and docosapentaenoic acid (22:5  $\omega$ -6) as unsaturated fatty acids (i.e. others are saturated fatty acids), it may be more advantageous to make pure DHA from this SCO than ordinary fish (containing many kinds polyunsaturated fatty acids as well as EPA and DHA).

# Appendix

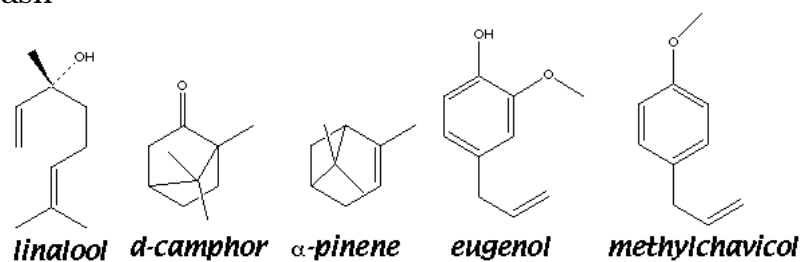
## Allspice



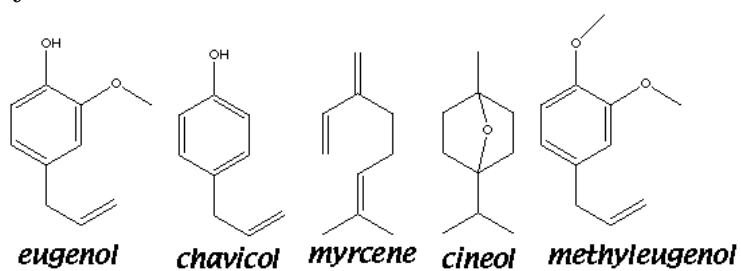
## Aniseeds



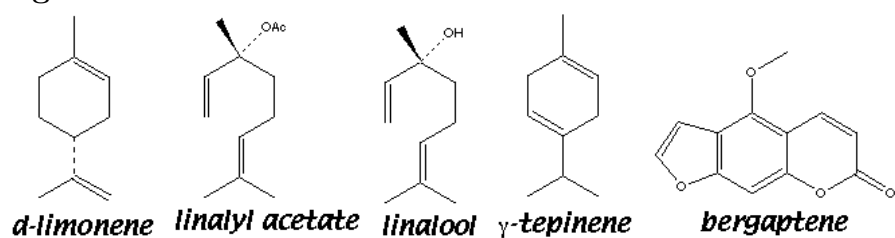
## Basil



## Bay leaf

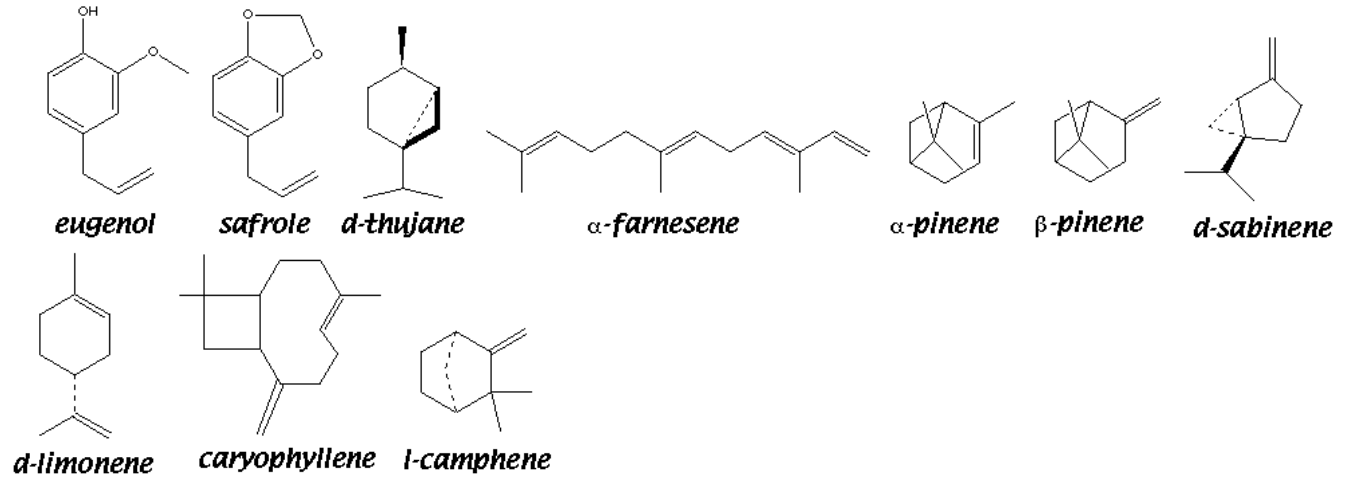


## Bergamot

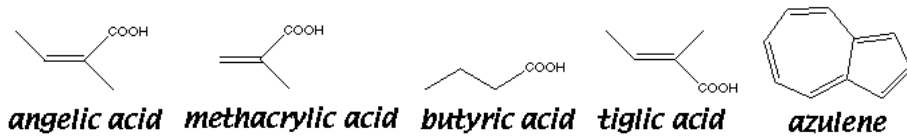


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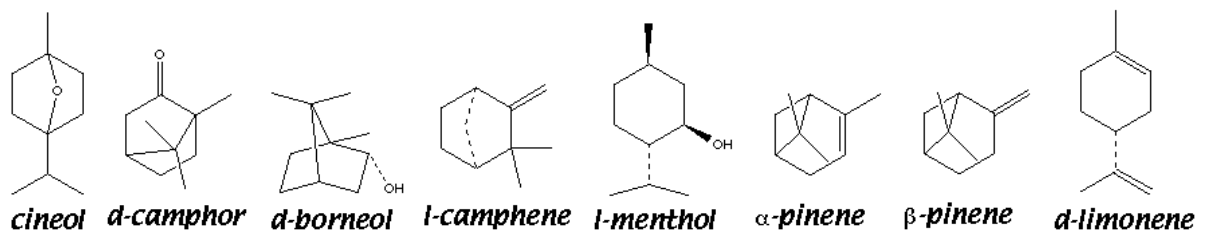
Black pepper



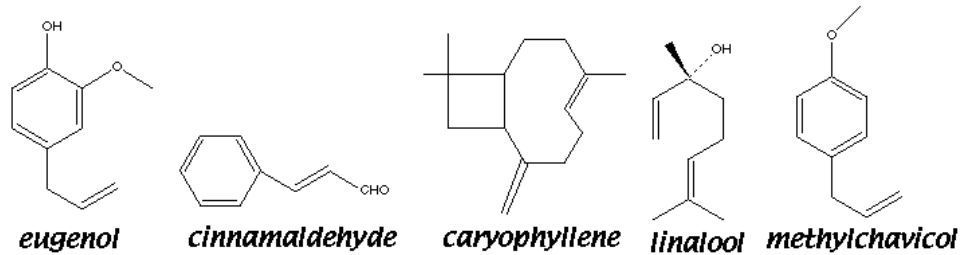
Chamomile



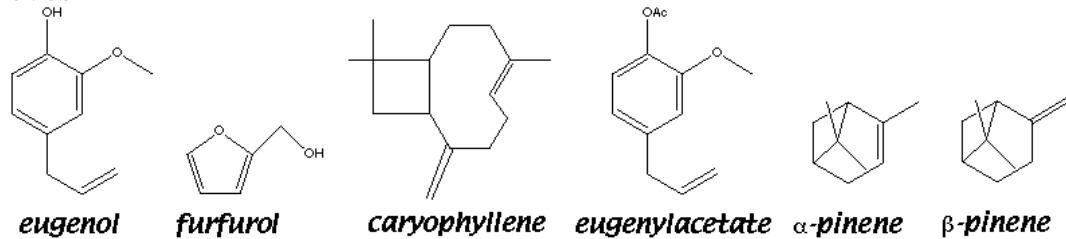
Camphor



Cinnamon

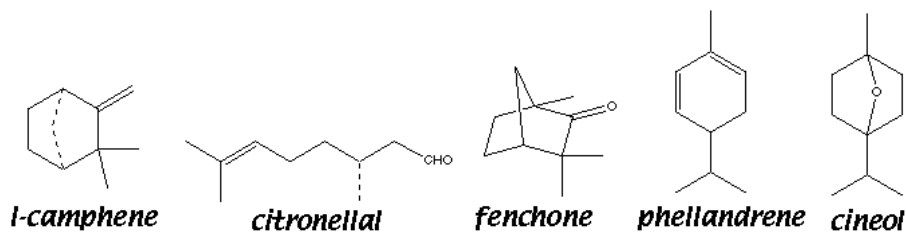


Cloves

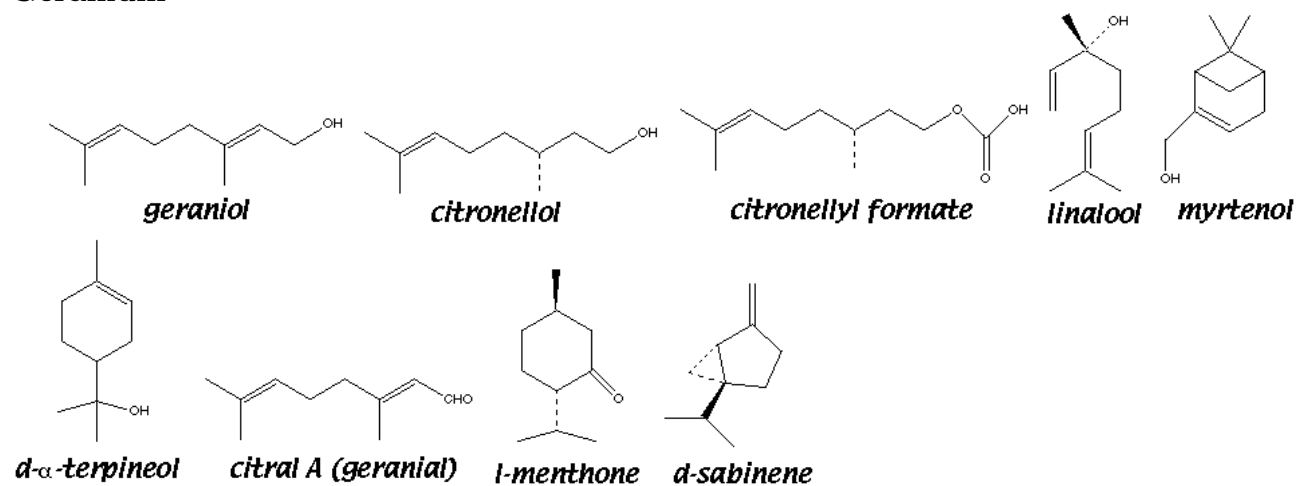


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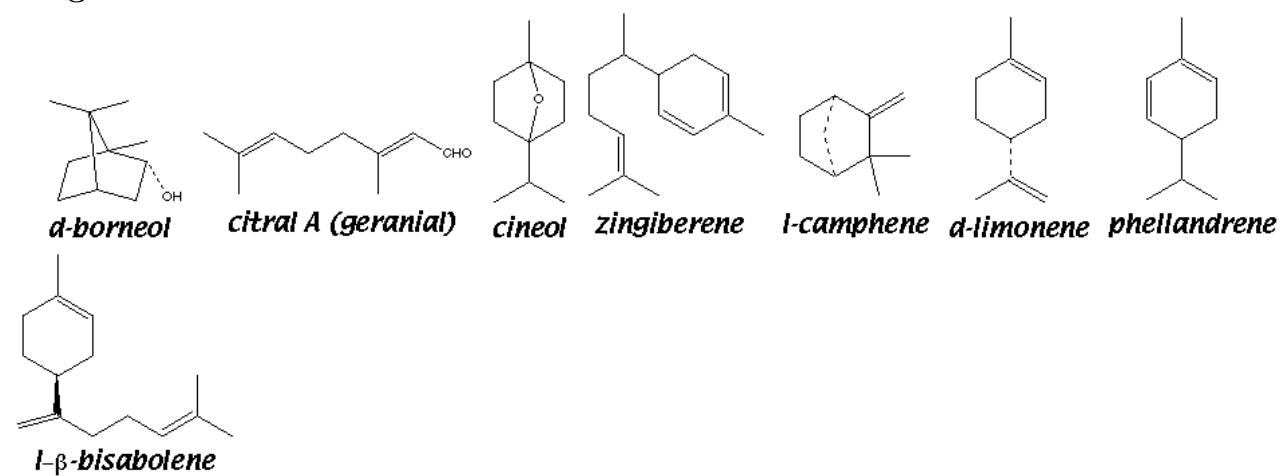
## Eucalyptus



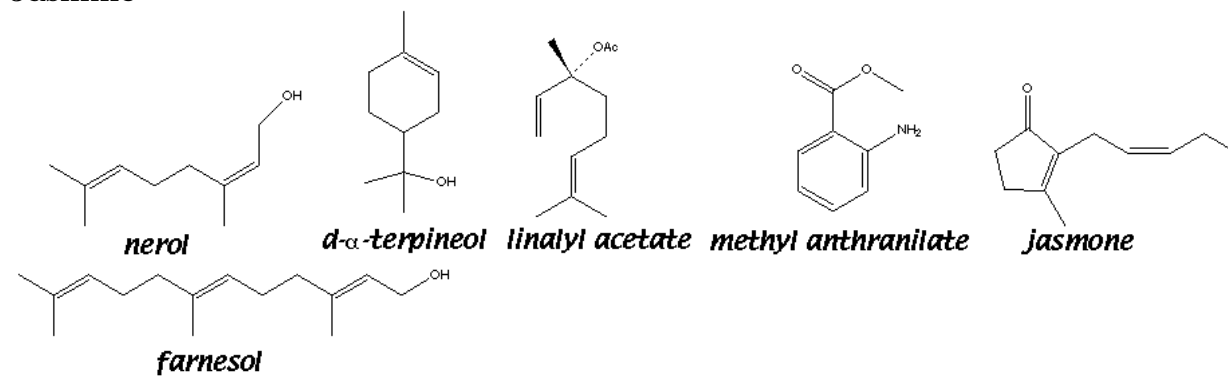
## Geranium



## Ginger

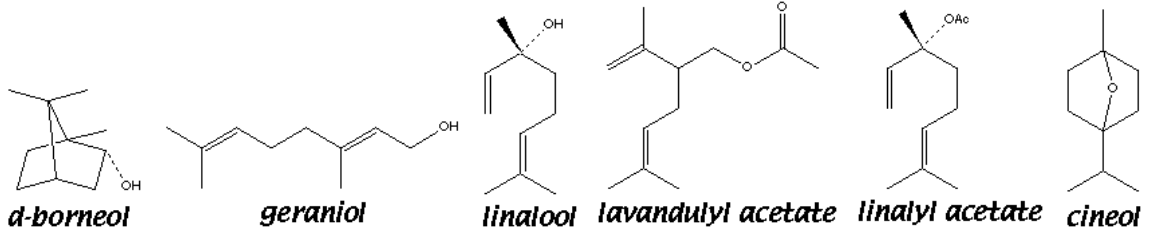


## Jasmine

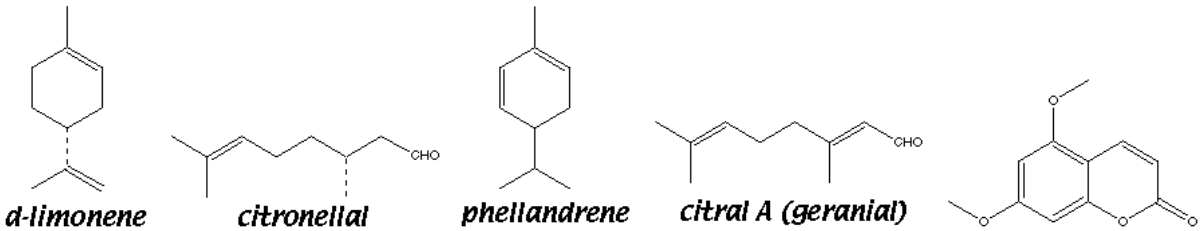


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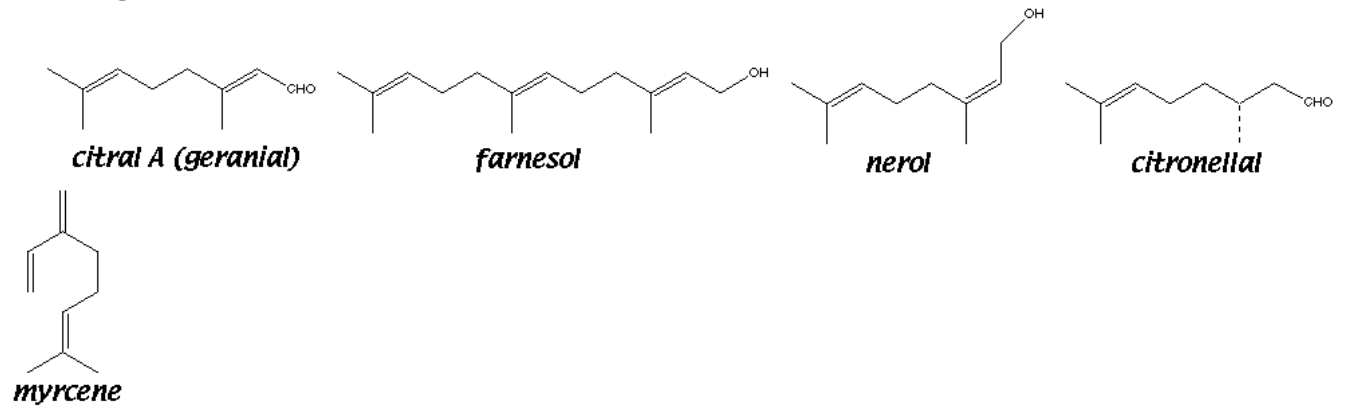
## Lavender



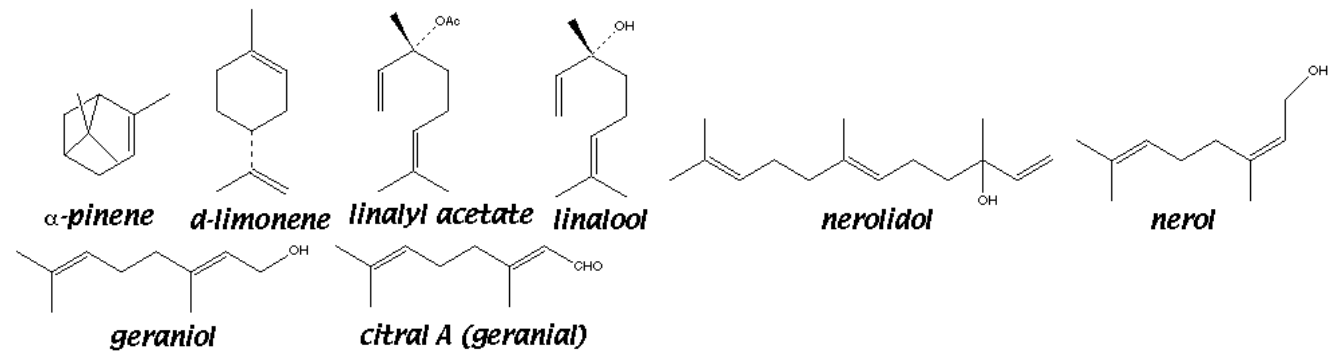
## Lemon



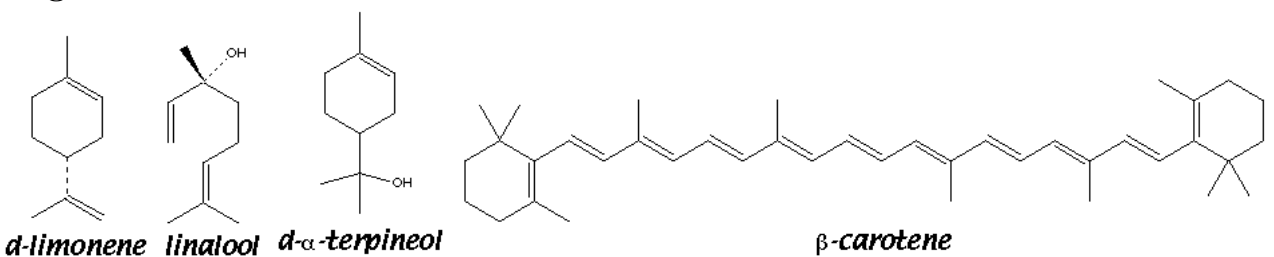
## Lemongrass



## Neroli

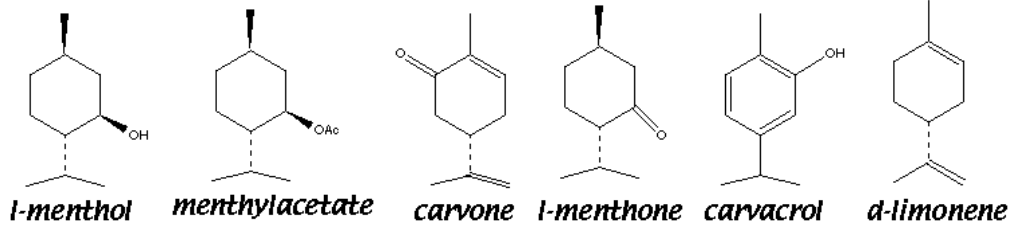


## Orange

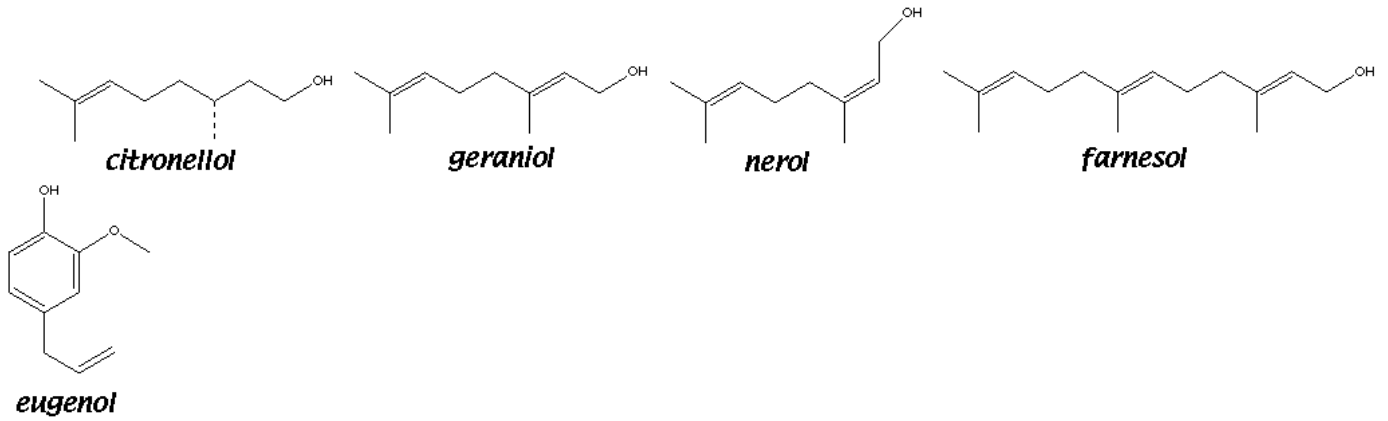


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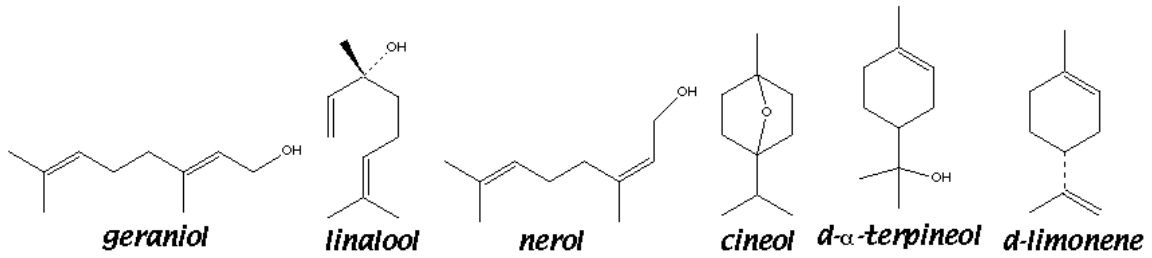
Peppermint



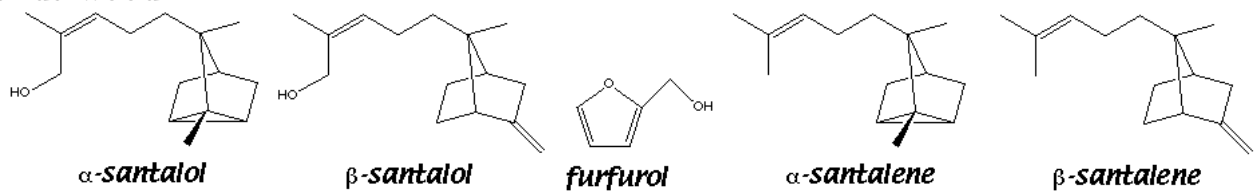
Rose



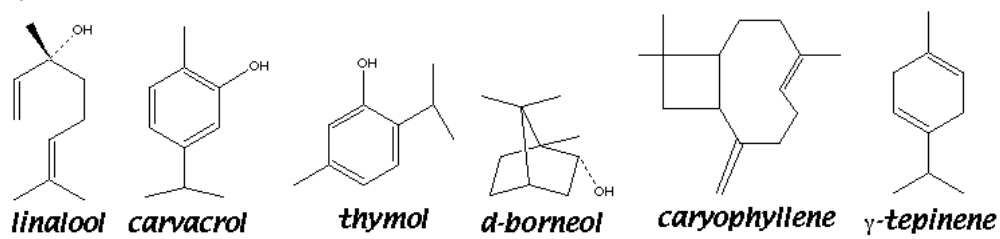
Rosewood



Sandalwood



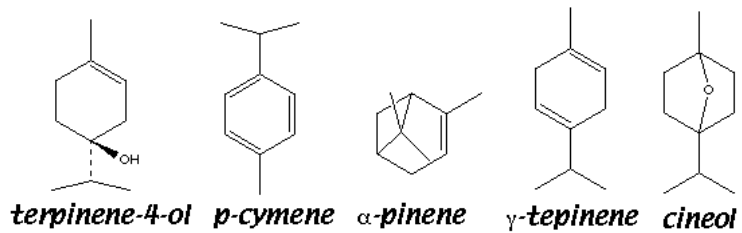
Thymes



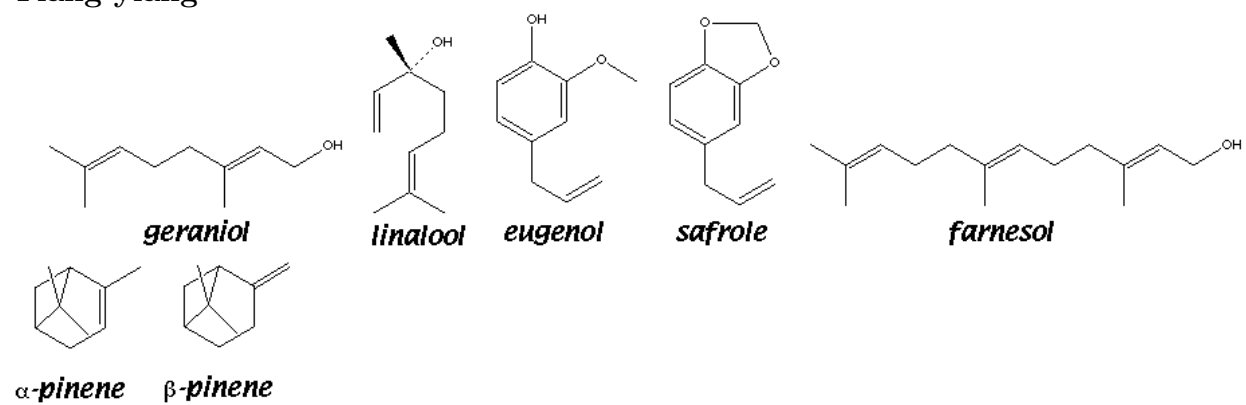
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Tea tree



Ylang ylang



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