CHAPTER 2

LITERATURE REVIEW

Aromatherapy is the practice of using volatile oils from plant, including essential oils, for psychological and physical well being. Aromatherapy is commonly associated with complementary and alternative medicine. Now it is popular used in spa. The word was coined in the 1920s by French chemist René Maurice Gattefossé.

2.1 Essential Oil

Essential oils are the odorous principles found in various plant parts. They are generated or secreted by specialized plant cells. When they are exposed to air at ordinary temperatures they evaporate, therefore they are called volatile oils, ethereal oils, or essential oils. The last term is applied because the oils represent the essences or odor constituents of the plants. They can be more or less fluid, are sometimes resinous and often have a coloring, which ranges from yellow to emerald green and blue to dark brownish red. As a result, they are usually colorless, particularly when fresh, but with age they may oxidize and resinify, thus becoming darker. Therefore, storage should be in a cool, dry place, tight stopper, preferably full in amber glass container [16].

Essential oils are mainly contained in the flowers and leaves. Besides this, they are also found in the wood, fruit, peel, bark, seed and root. Most plants contain essential oils but only the aromatic plants produce essential oil in sufficient quantities. These plants mainly belong to:

- The labiatae family (caraway, anise, fennel)

- The myrtaceae family (eucalyptus, cajuput, niaouli)
- The conifer family (pine, cedar, cypress, juniper)
- The rutaceae family (lime, lemon, orange, bergamot)
- The laurel family (cinnamon, borneol, sassafras)

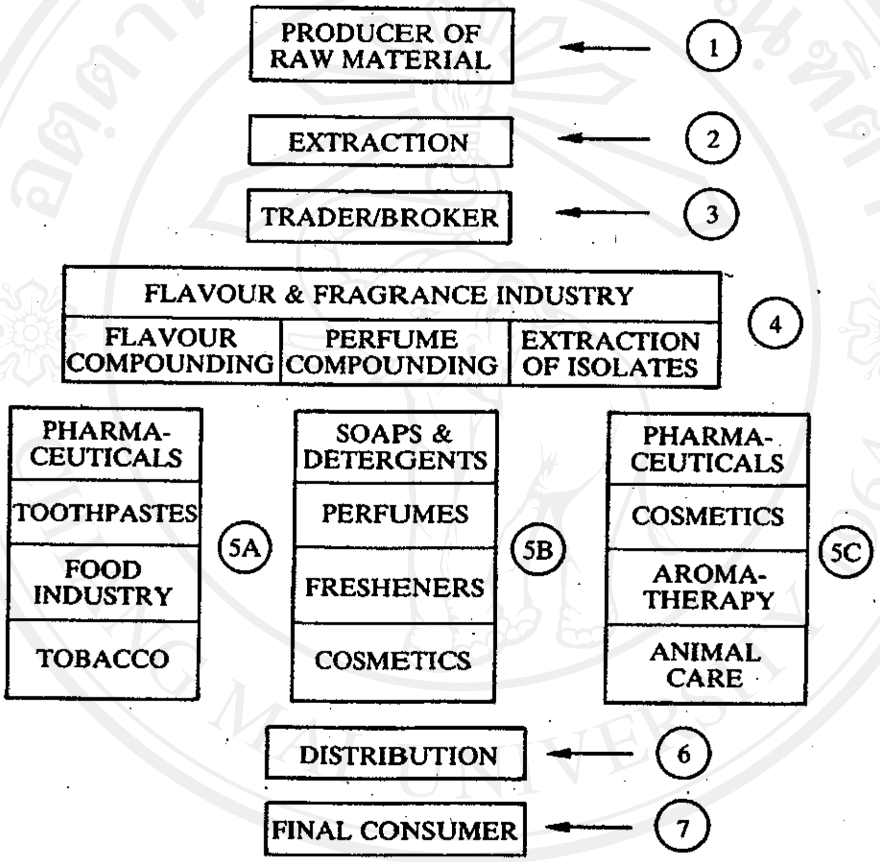


Figure 2.1 Routes of the production, trade and uses of essential oil

Uses

Most uses of essential oils are more down to earth and are part of everyday life. The use of essential-oil plants in fragrance materials is most important worldwide. There is a vast range of uses of fragrance products. The routes of the production, trade and uses of essential oil are shown in Figure 2.1 [17]. The most appealing use is in luxury perfumery. At present, essential oils are popular used in aromatherapy and spa. The main uses of perfumery are following [18].

1. Perfume industry for luxury perfumery such as perfume, eau de toilette, eau de cologne.
2. Cosmetic industry: cosmetic perfumery and house-cleaning products such as cream, lotion, shampoo, deodorant, soap, detergent.
3. Food and beverage industry such as sweets, ice cream, candy, chews gum, instant noodle, liqueur, and soft drink.
4. Pharmaceutical industry such as oral or dental hygiene, dental flosses; and active ingredient or flavoring agent in medicine.
5. Other industry such as cooking gas, plastics, etc.
6. Aromatherapy and alternative medicine.

Aromatherapy

Essential oils that are the pure essence of plant have been found to provide both psychological and physical benefits when used correctly and safely. It is important to note that perfume oils also known as fragrance oils or fragrances are not the same as essential oils. Perfume oils and fragrances contain unnatural chemicals and do not provide the therapeutic benefits of essential oils. Unfortunately, many companies improperly use the term aromatherapy on products that contain unnatural and perfume oils, so it is important to look at the ingredient label when seeking true aromatherapy products.

Essential oils stimulate the powerful sense of smell. It is known that odors we smell have a significant impact on how we feel. In dealing with patients who have lost the sense of smell, doctors have found that a life without fragrance can lead to high incidence of psychiatric problems such as anxiety and depression [19-21]. We have the capability to distinguish 10,000 different smells. It is believed that smells enter through cilia (the fine hairs lining the nose) to the limbic system, the part of the brain that controls our moods, emotions, memory and learning [22-27]. The pathways of volatile oil or essential oil into the body are shown in Figure 2.2[28].

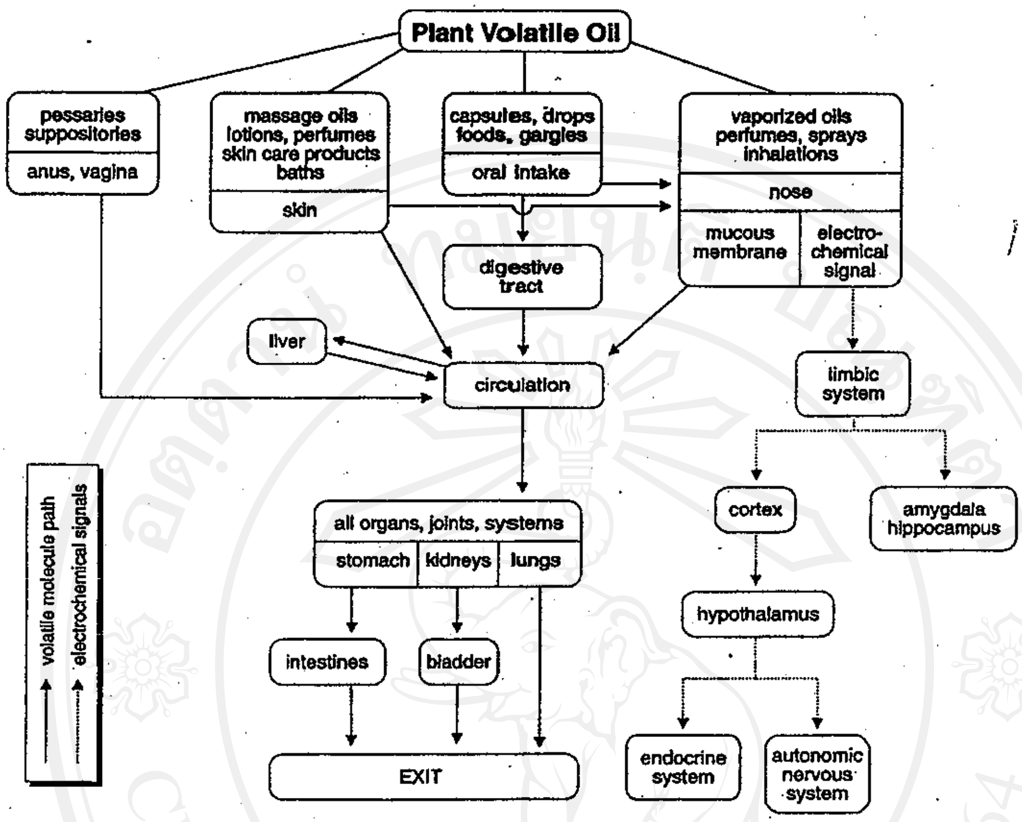


Figure 2.2 Pathways of volatile oil or essential oil into the body

Studies with brain wave frequency have shown that smelling lavender increase alpha waves in the back of the head, which are associated with relaxation. Fragrance of Jasmine increases beta waves in the front of the head, which are associated with a more alert state.

Scientific studies have also shown that essential oils contain chemical components that can exert specific effects on the mind and body. Their chemistry is complex, but generally includes alcohols, esters, ketones, aldehydes, and terpenes. The effects of these chemical components are summarized in the accompanying Table 2.1 [13, 23, 29, 30].

Table 2.1 The effects of chemical components of essential oils

Name	Properties	Essential oils
Aldehydes	Anti-inflammatory, calming, sedative, anti-viral.	Lemon balm, lemon grass, eucalyptus, citronella
Alcohols	Bactericidal (kills bacteria), stimulant, energizing, vitalizing, antiviral, diuretic.	Rose, petitgrain, rosewood, peppermint, sandalwood, tea tree, myrtle, patchouli, ginger
Phenols	Strongly bactericidal, tonic, stimulates immune system, invigorating, warming	Clove, cinnamon, thyme, oregano, savory, cumin
Ketones	Wound healing, mucolytic (eases the secretion of mucous), stimulates new cell growth.	Eucalyptus globulus, sage, camphor, rosemary, hyssop
Terpenes	Very stimulating, potential skin irritants, anti-viral properties.	Lemon, orange, angelica, bergamot, black pepper, nutmeg, pine oil
Sesquiterpenes	Anti-phlogistic, bacteriostatic, anti-inflammatory, sedative, anti-viral, anti-carcinogenic, immune stimulant.	Blue chamomiles, tansy, immortelle, yarrow, tagetes
Esters	Anti-fungal, sedative, calming, spasmolytic, fungicidal, anti-inflammatory.	Roman chamomile, lavender, clary sage, petitgrain, bergamot
Lactones	Anti-inflammatory, mucolytic.	Arnica, elecampane.
Ethers	Stimulant, harmonizing to the nervous system, spasmolytic, antiseptic, expectorant (increases secretions), diuretic.	Cinnamon, clove, anise, basil, tarragon, parsley, sassafras

Each essential oil contains as much as 100 chemical components, which together exert a strongly effect on the whole person. Depending on which component is predominating in oil, the oils act differently. For example, some oils are relaxing, some soothes you down, some relieves your pain, etc. Then there are oils such as lemon and lavender, which adapt to what your body needs, and adapt to that situation (These are called "adaptogenic"). The mechanism in which these essential oils act on us is not very well understood. What is understood is that they affect our mind and emotions. They leave no harmful residues. They enter into the body either by absorption or inhalation.

Although essential oils are natural products, it is still necessary to follow certain precautions when using them. It is advisable that the public exercises caution and those they consult a qualified aromatherapy practitioner before using aromatherapy for medical use. Essential oils are very concentrated and volatile. Improper use may cause burns, allergic reactions, headache, or nausea. To avoid complications directions should be closely followed and certain safety guidelines should be followed.

- Essential oils are contraindicated in asthma, heart conditions, hypertension, cancer, epilepsy, and frequent allergic reactions.
- Essential oils are contraindicated in pregnancy due to the potential toxicity to the mother and fetus and the risk of triggering spontaneous abortion.
- Avoid using essential oils on babies and children less than 5 years of age. Use aromatherapy on children older than 5 years with caution.
- Essential oils must always be diluted according to the instructions before applying to the skin or using for inhalation.
- Before using an essential oil a simple patch test must be done to ensure it will not irritate the skin. Any reaction will be immediate.
- Toxicity and contraindications for each should be well understood.
- Keep essential oils away form eyes and mucous membranes.
- Due to the strong association between smell and memory, special care should be taken when using aromatherapy on patient's undergoing chemotherapy or feeling

very ill. The smell of the oil may induce nausea, vomiting or negative emotions in subsequent context.

- High doses (10-20 ml) of some oils may cause non-lethal toxicity. These include wintergreen, sage, aniseed, thyme, lemongrass, fennel, clove, cinnamon, camphor, and cedar wood.
- Certain oils are more commonly associated with allergic reactions including basil, fennel, lemongrass, rosemary, and verbena oils.

Some essential oils which you should be cautious about:

The following list is incomplete because there is a never-ending flow of 'new' oils being pushed into aromatherapy. It is common to find that scientists have found very good therapeutic properties in some newly discovered plant oil. These scientists then appear at International conferences to talk about their findings. The next thing of course is everyone wants to buy this new magical oil. No one, the scientists concerned included, pause to give a second thought to the potential side effects of this wonderful new discovery. Unfortunately it is not until some poor individual has been harmed that someone gives safety a second thought. So the moral of all this is to be safe, stick to those oils the safety of which has been well documented [13, 23].

- **Amni visnaga:** No formal safety trials are known.
- **Benzoin resinoid and oil:** A well documented sensitiser - RIFM recommend only grades processed to remove the allergens should be used in consumer products. These grades are not generally available via aromatherapy suppliers.
- **Bergamot oil** expressed**:** A potent photosensitiser
- **Calamus oil:** A potential carcinogen, and banned in cosmetics.
- **Catnip:** No formal safety trials are known.
- **Cinnamon bark oil:** An extremely powerful irritant and an even worse sensitiser.
- **Chamomile moroc:** Often labeled just as "Chamomile oil". This oil has not undergone internationally acceptable safety testing.

- **Copaiba or copahu:** Can cause sensitisation reactions if it is old and oxidised.
- **Eucalyptus chemotypes:** The only types that have been tested are the globulus types and E. citriodora.
- **Inula graveolens:** Related species Inula *helenium* root oil, is one of the most hazardous essential oils available. If the graveolens variety contains the same sensitising agents is not known. Therefore, until such time as formal testing has been done, it is most unwise to use this oil on the skin.
- **Kanuka:** This oil has not undergone internationally acceptable safety testing.
- **Manuka:** This oil has not undergone internationally acceptable safety testing.
- **Niaouli:** The pure oil has not undergone internationally acceptable safety testing.
- **Peru balsam:** A very powerful sensitiser. RIFM recommend "not to be used as a fragrance ingredient".
- **Ravensara aromatica and other ravensara oils:** None of these oils have undergone any formal safety trials. There also seems to be a lot of manufactured oil on the market, which makes their safety, suspect.
- **Rosemary chemotypes:** Only the common cineol types have been tested.
- **Rue oil:** A terrible photosensitiser and sensitiser. It never uses this oil.
- **Sassafras:** This oil is restricted to such low levels in cosmetic products throughout Europe, that it effectively bans its use. Tests have shown it is possibly carcinogenic.
- **Spikenard:** Like its cousin valerian, these oils have not undergone internationally acceptable safety testing.
- **Tagetes** (sometimes mis-described as calendula): A powerful photosensitiser - RIFM say a no effect level is 0.05%. Therefore to use it on skin exposed to the light would be foolish.
- **Tansy oil:** Extremely toxic, and of little if any use in aromatherapy.
- **Thyme chemotypes:** Only the common phenol types have been tested.
- **Verbena oil:** An extremely powerful sensitiser - recommended by the RIFM "not for use as a fragrance ingredient". Massive percentages of adverse skin

reactions are recorded from testing a whole range of verbena oils. There also seems to be a lot of manufactured oil on the market, which makes their safety, suspect.

- **Wormseed** (Chenopodium): Extremely toxic. Banned from general sale in the UK because of the deaths reported from its consumption in the past.
- **Yarrow:** Another essential oil that has not undergone formal safety testing.

If anyone sees such oils on open sale without appropriate warnings, you should tell the vendor that the safety is unknown. Often they may not know, and may have relied for their knowledge entirely on the popular aromatherapy novels, on some of the appallingly poor training courses around, or con artist oil salespeople.

Properties

1. Physicochemical Property

Although differing in their chemical constitution, they have many physical properties in common [13, 16, 18, 27, 31].

a. Essential oils are liquids, some are resinous or solids.
b. They are usually colorless and often have a coloring, which ranges from yellow to emerald green and blue to dark brownish red, but with age they may oxidize and resinify, thus becoming darker.
c. They are highly volatile at room temperature with characteristic odors.
d. Most of them are non-oily and usually lighter than water.
e. They are insoluble in water, however, will impart odor to water.
f. Their viscosities are low.
g. They are soluble in alcohol, ether, fixed oils (vegetal), organic solvents.
h. They dissolve grease, iodine, sulfur and phosphorous; reduce certain salts.
i. Their boiling points vary from 160° to 240°C.
j. Their densities vary from 0.759 to 1.096.
k. They have high refractive indices.

l. Most of them are optically active, specific rotation is often a valuable diagnostic property mixture of many constituents.

m. Essential oils are diffuse and penetrating. Their high degree of vibratory capacity gives them this quality.

2. Biological property

Essential oils not only give the plant their scent, but they are also essential for the plant biological processes. Because of this many refer to the oils as the life force of the plant. There are in excess of 70 essential oils in use for the purpose of aromatherapy today. Each has its own characteristic aroma and profile of therapeutic effects. Some are soothing, some are invigorating, some are believed to relieve mental conditions such as depression and anxiety, some are thought to have direct physical effects on organ systems, and still others are thought to possess antibiotic, antiseptic, antibacterial, antiviral, antifungal, and anti-inflammatory properties. Those, which are implemented for the treatment of pain, include lavender, peppermint, rosemary, eucalyptus, marjoram, geranium, marigold and others [31].

3. Chemical components

Essential oils are complex mixtures of sometimes hundreds of chemical compounds: terpenes, phenols, alcohols, ketones, esters, phenolic ethers, aldehydes, oxides, lactones, acids, etc. Many of characteristic components of essential oils are classified in to 4 groups as follow [14, 18, 27, 31].

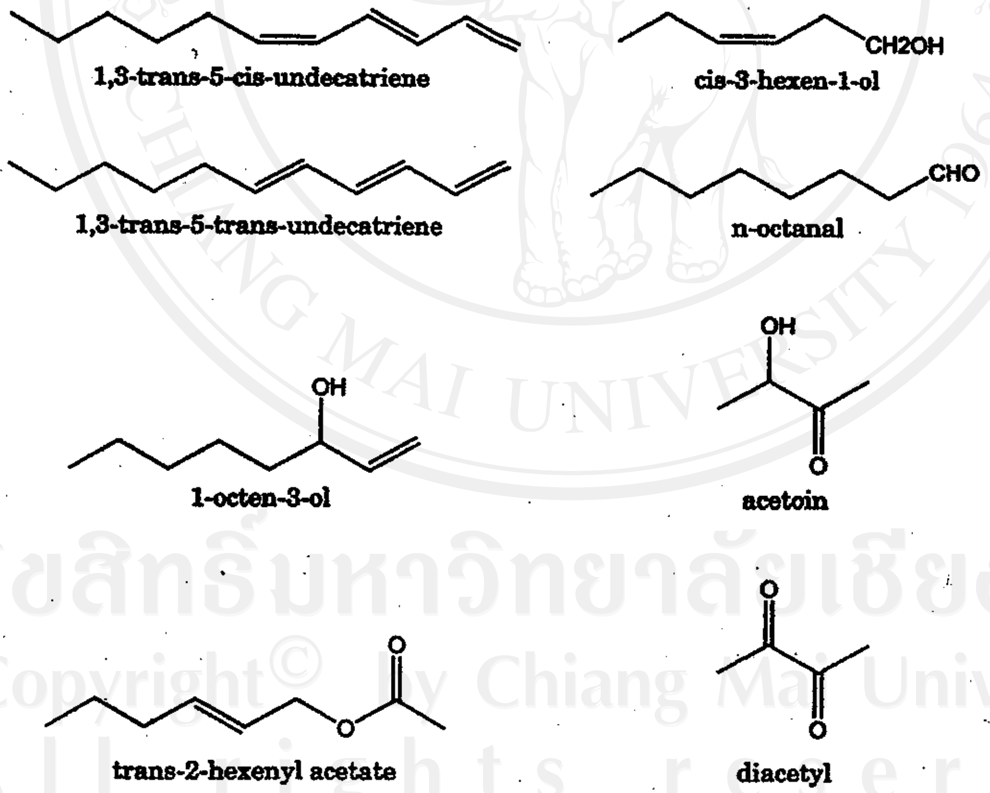
1. Aliphatic compounds

Aliphatic compounds are acyclic organic compounds. The chain of C-atoms in these compounds may be straight or branched and some of the bonds between C-atoms may be unsaturated.

- Aliphatic hydrocarbons occur abundantly in foodstuffs such as fruit, but contribute to their odor to a limited extent only, e.g. 1,3-*trans*-5-*cis*-

undecatriene and 1,3-*trans*-5-*trans*-undecatriene, the significantly odor of galbanum oil

- Aliphatic alcohols odor is general weak and their role as components in fragrance compositions is limited. An exception is 3-octanol, occurring in mushrooms and imparting their characteristic smell.
- Aliphatic aldehydes are important compounds in perfumery and flavoring. The series n-octanol, n-nonanol, n-decanol and n-undecanol, for instance, occur in citrus oils.
- Aliphatic esters are important flavor and fragrance compounds occurring widely in nature. In perfumery, acetates of alcohols up to C_6 are used for their fruity notes, C_8. C_{12} acetates for their blossom fragrance. Structural formulas of some aliphatic fragrance compounds are given in Figure 2.3.



1,3-trans-5-cis-undecatriene

cis-3-hexen-1-ol

1,3-trans-5-trans-undecatriene

n-octanal

1-octen-3-ol

acetoin

trans-2-hexenyl acetate

diacetyl

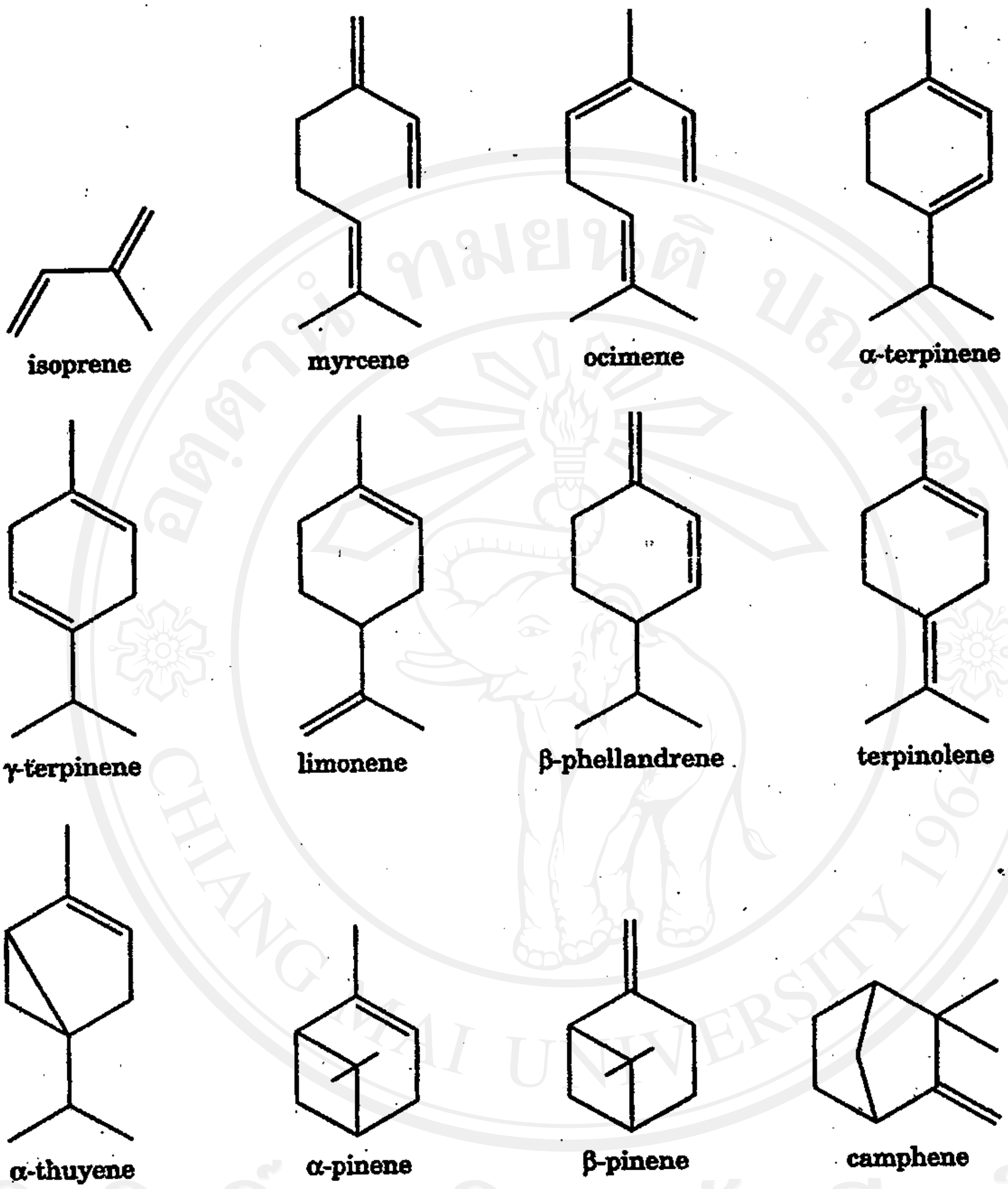
Figure 2.3 Structural formulas of some aliphatic fragrance compounds

2. Terpenes and terpene derivatives

Terpenes constitute a widely represented group of substances. Although they show various structural diversity, they share a common characteristic: they are built from 2 (monoterpenes), 3 (sesquiterpenes) or more isoprene (C_5H_8) units. Isoprene is one of the basic compounds in animal and plant biomolecules. It is formed from acetyl-CoA that plays a role in the synthesis and oxidation of sugars. The terpene hydrocarbon contributes to the odor and taste of essential oil to a limited extent only, but their oxygenated derivatives are among the most important aroma chemicals.

- Monoterpenes conform to molecular formula $C_{10}H_{16}$ and can be acyclic, monocyclic, bicyclic or tricyclic. Cyclic monoterpenes occur in essential oil, sometimes in considerable amounts. By themselves they generally contribute relatively little to the odor of a fragrance or flavor products, but often serve as starting materials for the biological or chemical flavor and fragrance compounds, e.g. α-terpinene, γ-terpinene, limonene, α-phellandrene, β-phellandrene and terpinolene. Of the bicyclic terpenes, the α-pinene and β-pinene are technologically the most important by far. Structural formulas of some monoterpenes are given in Figure 2.4.

- Sesquiterpenes are compounds generated from 3-isoprene units and conforming to the formula: $C_{15}H_{24}$. As there are so many, it is impossible to generalize about their molecular structure. Many sesquiterpenes are bicyclic, having two C_6-rings or a C_6 and a C_5 ring. Structural formulas of some sesquiterpenes are given in Figure 2.5.



isoprene

myrcene

ocimene

α-terpinene

γ-terpinene

limonene

β-phellandrene

terpinolene

α-thuyene

α-pinene

β-pinene

camphene

Figure 2.4 Structural formulas of some monoterpenes

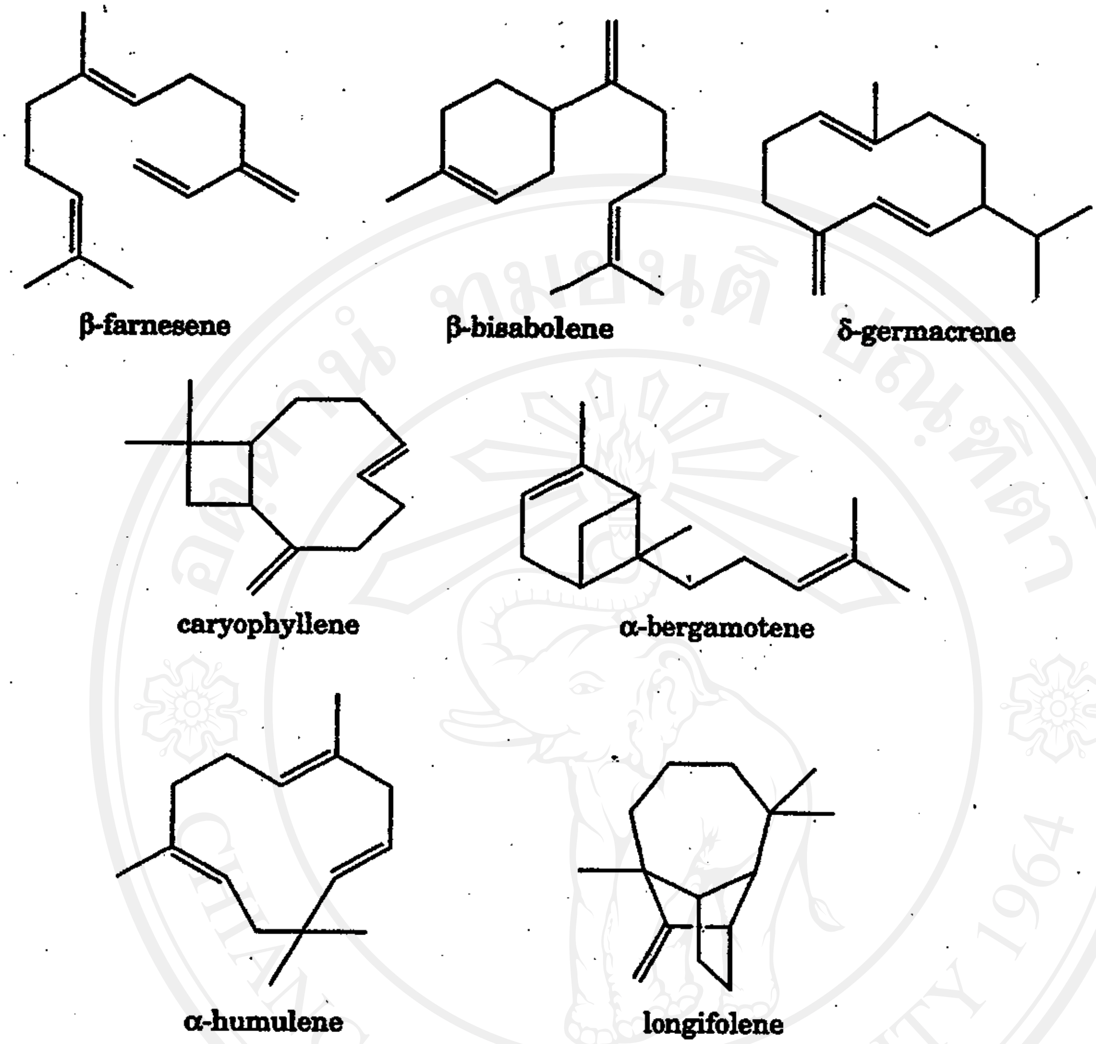


Figure 2.5 Structural formulas of some sesquiterpenes

- Oxygenated derivatives of monoterpenes and sesquiterpenes are more important than the terpene hydrocarbons as aroma chemical. The characteristic odor of many essential oils is representative of the combined odors of the oxygenated compounds. Important groups of oxygenated compounds are alcohols, aldehydes, ethers, ketones, acids and esters. Acyclic monoterpenes alcohols and acyclic sesquiterpenes alcohols occur in many essential oils and contribute strongly to their characteristic odor, e.g. citronellol, geraniol, linalool and nerol.

Structural formulas of several important acyclic terpene alcohols are given in Figure 2.6. The most important aldehydes derived from acyclic monoterpenes and sesquiterpenes are citral and citronellal.

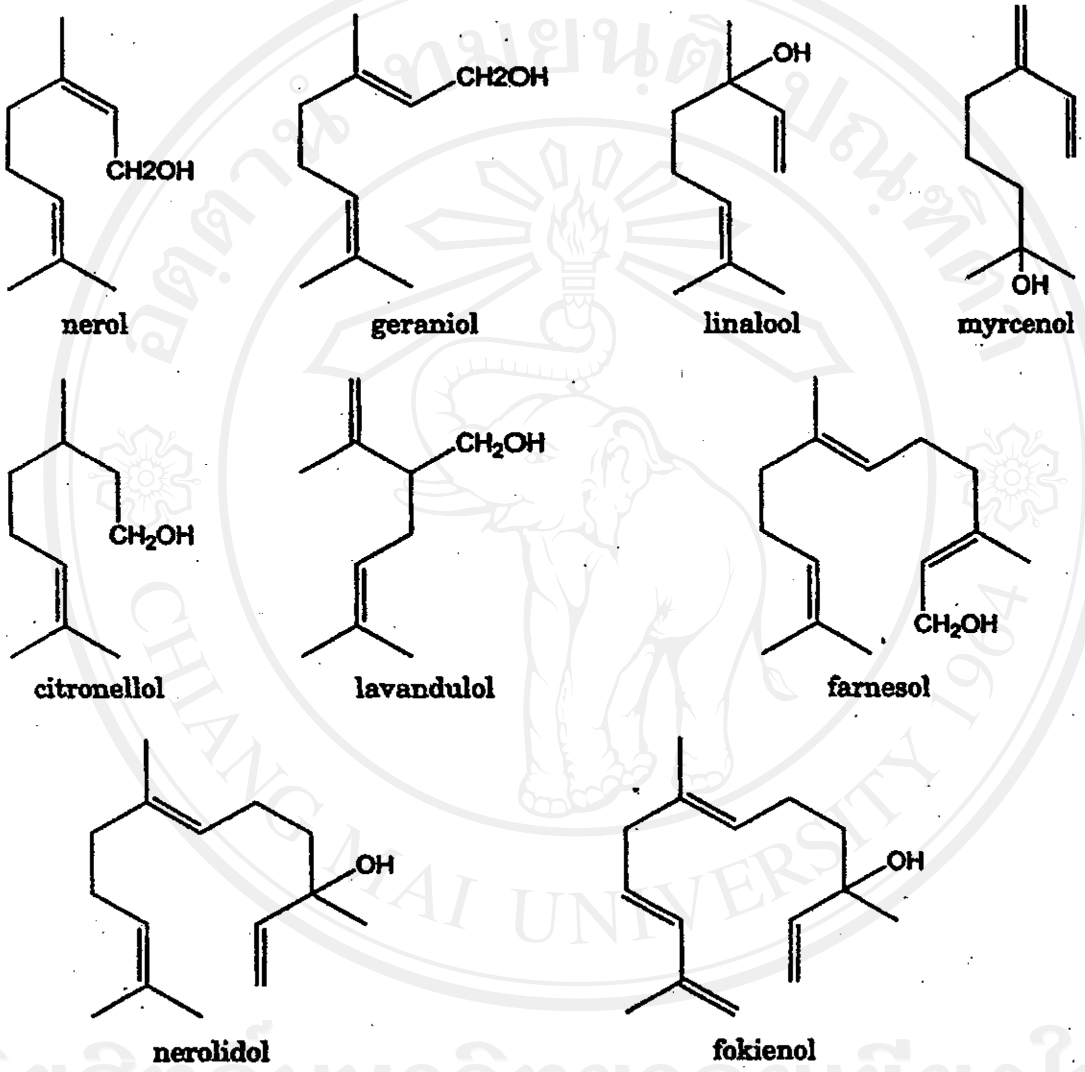


Figure 2.6 Structural formulas of several important acyclic terpene alcohols

3. Benzene derivatives

In chemistry, benzene derivatives or benzenoids (often confusingly named aromatic compounds) are compounds containing a characteristic benzene ring, often represented as a C_6 ring with 3 double bonds alternating with single bonds between the C-atom. It is very large and varied group that includes many natural and synthetic flavor and fragrance compounds. The most important hydrocarbon derived from benzene is p-cymene; it occurs in many essential oils and has a weak citrus odor when pure. Esters of aromatic alcohols and aliphatic acids are of interest in flavors and fragrances because of their characteristic odor properties such as benzyl acetate is the main component of jasmine oil and gardenia oil. Some benzenoid fragrancecompounds are represented in Figure 2.7.

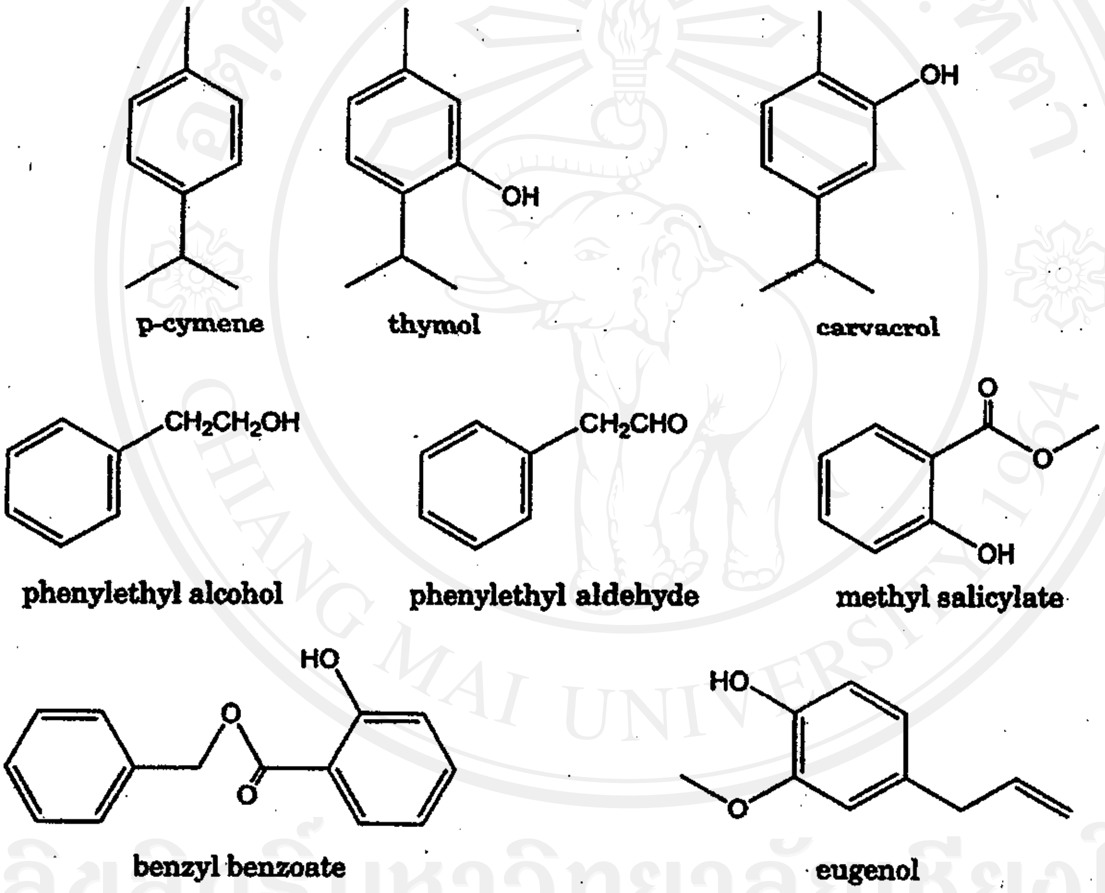


Figure 2.7 Some benzenoid fragrance compounds

4. Miscellaneous compounds

Several nitrogen and sulphur compounds impart characteristic sensory properties to essential oils, even when they are in very small amount. Their structural formulas are given in Figure 2.8.

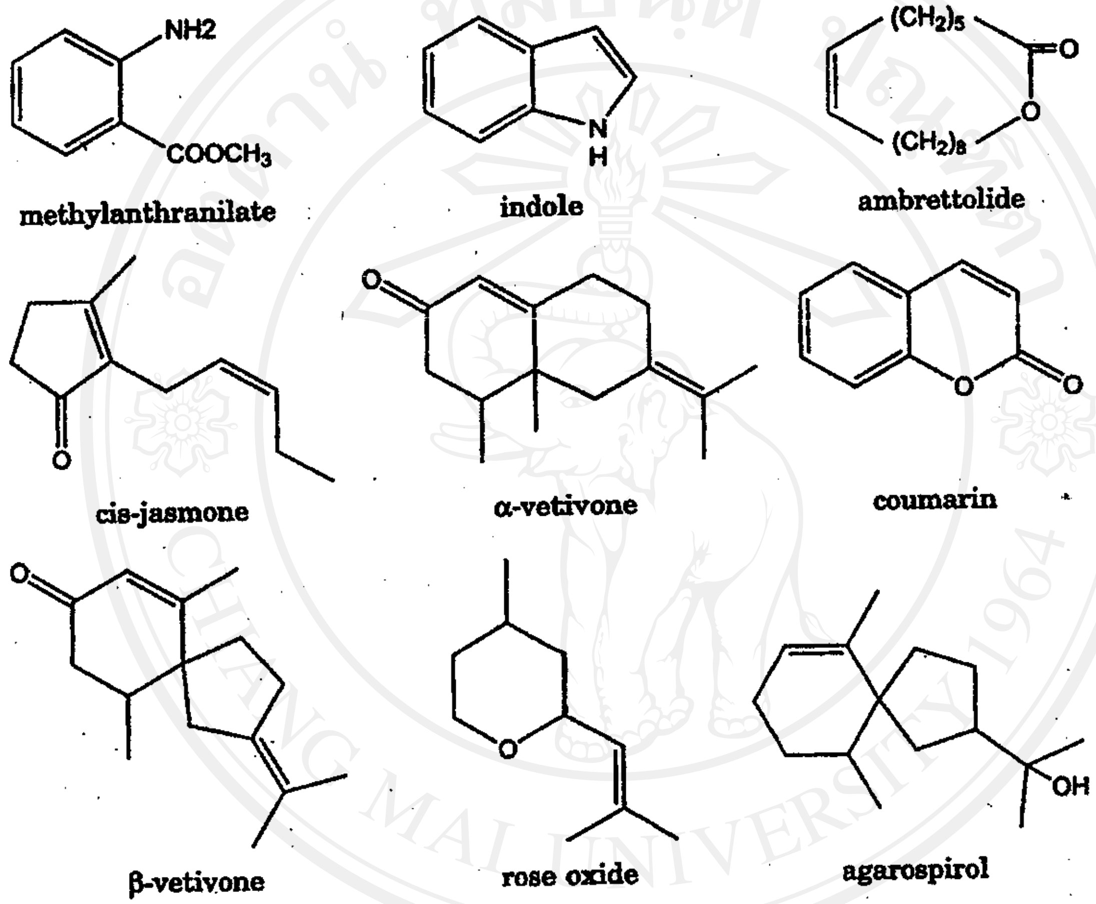


Figure 2.8 Structural formulas of Miscellaneous compounds

Extraction

Essential oils can be extracted using a variety of methods, although some are not commonly used today. Currently, the most popular method for extraction is steam distillation, but as technological advances is made more efficient and economical methods being developed [14, 18, 27, 31].

1. Distillation

The vast majority of true essential oils are produced by distillation. There are different processes used, however. In all of them, water is heated to produce steam, which carries the most volatile chemical of the aromatic material with it. The steam is then chilled (in a condenser) and the resulting distillate is collected. The essential oil will normally float on top of the Hydrosol (the distilled water component) and may be separated off.

2. Steam Distillation

True Steam distillation uses an outside source of steam, which pipes the steam into the distillation unit, sometimes at high pressure. The steam passes through the aromatic material, and exits into the condenser. To extract the essential oil, the 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 essential oil vapor and the steam then pass out 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 and floats to the top.

3. Hydro distillation

The botanicals are fully submerged in water, producing a "soup", the steam of which contains the aromatic plant molecules. This is the most ancient method of distillation and the most versatile. It's the method most often used in primitive countries. The risk, of course, is that the still can run dry, or be overheated, burning the aromatics and resulting in an essential oil with a burnt smell. Hydro distillation seems to work best for powders (i.e., spice powders, ground wood, etc.) and very tough materials like roots, wood, or nuts.

4. Water & steam Distillation

A water and steam distillation arrangement can be compared to a kitchen steamer basket, with the botanicals supported in a "basket" over boiling water, thus exposing the plant material only to the rising steam vapors. This is the best method for distilling leafy materials, but doesn't work well for woods, roots, seeds, etc.

5. Enfleurage

The enfleurage method is to use a fatty substance to absorb the essential oil, which is then separated from the substance. This method is suitable for cosmetic products since the fats can be processed into ointments, creams etc.

6. Maceration

It actually creates more of infused oil rather than an essential oil. The plant matter is soaked in vegetable oil, heated and strained at which point it can be used for massage. Maceration is similar to enfleurage. Maceration is used to extract essential oils from animal ingredients, vanilla and iris. These materials are steeped into vats of oil until the scented parts dissolve. The oil may be heated to speed up the process. Maceration takes long periods of time (sometimes years).

7. Cold Pressing

We are all familiar with the spray of orange essential oil that can be released by scoring or zesting the skin of the fruit. The cold pressed citrus oils are commercial produced just this way, by machines which score the rind and capture the resulting oil. Although many citrus oils are also produced by steam distillation, they seem to lack the vibrancy of the cold pressed oils. Cold pressing is used to extract the essential oils from citrus rinds such as orange, lemon, grapefruit and bergamot. The rinds are separated from the fruit, are ground or chopped and are then pressed. The result is a watery mixture of essential oil and liquid which will separate given time.

It is important to note that oils extracted using this method have a relatively short shelf life, so make or purchase only what you will be using within the next six months.

8. Solvent Extraction

A hydrocarbon solvent is added to the plant material to help dissolve the essential oil. When the solution is filtered and concentrated by distillation, a substance containing resin (resinoid), or a combination of wax and essential 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. This is not considered the best method for extraction as the solvents can leave a small amount of residue behind which could cause allergies and effect the immune system.

9. Carbon Dioxide Extraction

When CO_2 (carbon dioxide) is subjected to high pressure, the gas turns into liquid. This liquid CO_2 can be used as a very inert, safe, "liquid solvent." which will extract the aromatic molecules in a process similar to that used to extract absolutes (above.) The advantage, of course, is that no solvent residue remains, since at normal pressure and temperature, the CO_2 simply reverts to a gas and evaporates. CO_2 extraction has given us essences of some aromatics that don't yield essential oils, Rose Hip Seed, and Calendula, for examples. If the same essential oil is available both as a steam distilled essential oil and a CO_2 extracted essence, the CO_2 seems to have a richer, more intense scent, since more of the aromatic chemicals are released through this process.

10. Florasols/Phytols

This extraction method uses a new type of benign gaseous solvents. In the late 1980s Dr. Peter Wilde first recognized the unique properties of these solvents for the extraction of aromatic oils and biologically active components from plant materials, for use in the food, pharmaceutical, aromatherapy and perfume industries. "Florasol" (R134a), is the solvent upon which the process is based Extraction occurs at or below ambient temperatures; hence there is no thermal degradation of the products. The extraction process utilizes the selectivity of the solvent and produces free flowing clear oil free of waxes.

Essential oil Quality

Quality of essential oil are studied from appearance, physical properties and chemical components and adulteration .The United State Pharmacopoeia (USP), The

European Pharmacopoeia (EP) and The British Pharmacopoeia (BP) issue testing of properties as follow [13, 18, 31].

1. **Appearances**

   Such as status, color, odor, taste, etc.

2. **Physical properties**
   - Specific gravity
   - Relative density (d_{20}^{t})
   - Refractive index (n_D^{t})
   - Optical rotation: angle of optical rotation (α_D^{t}) or specific optical rotation (α_D^{t})
   - Solubility in ethanol

3. **Chemical components**
   - Acid value
   - Ester value
   - Carbonyl value
   - Ketone value
   - 1,8-cineole content
   - Foreign esters in essential oils
   - Residue on evaporation
   - Water in essential oils
   - Thin Layer Chromatography (TLC)
   - Gas Chromatography (GC)

Effect Factors on Essential oil

Quantity and quality of essential oil are vary from many factors as [20, 23]:

- Kinds of plant
- Heredity (genotypes and phenotypes)

- Growth environment: temperature, humidity, totals duration of daylight, wind flow, climate, features of the land, country
- Ontogeny and cultivation time
- Parts of plant
- Characteristics of material
- Methods of extraction
- Storage

Quality control

Until a few decades ago, the human nose, supported by the measurement of a number of physical characteristics and a few chemical analyses, was the chief mean of verifying the density, purity and naturalness of essential oils. The development of capillary gas chromatography/mass spectrometry (GC/MS) methods has greatly improved knowledge of the chemical composition of essential oils. Currently, compounds present in as little as 1 ppb (part per billion) can be detect and identified, making it easier to distinguish between a pure natural essential oil and oils which foreign compounds have been added. As both the pathways of synthesizing chemical compounds and the by-products of these processes are well known, demonstration of their presence in an essential oil can be proof that the oil has been adulterated.

Although this method has made it easier to detect adulteration with synthetics, it cannot reveal adulteration-involving compounds derived from cheap natural linalool and pinene. The measurement of nuclear magnetic resonance (NMR) of ^{13}C and ^{12}C has been refine to such extent that it can identify whether a compounds originates from a C_3 or C_4 plant.

The newest way to characterize chemical compounds is a method based on deuterium nuclear magnetic resonance spectrometry. It appears that the ^{2}H or deuterium is very unevenly distributed over the various bonds in a molecule. As the internal distribution varies with the origin of the molecule, this method can distinguish if e.g. anethol is prepared from star anise, estragol or petroleum [18, 31].

Method of analysis [18, 32]

1. Thin Layer Chromatography (TLC)

Thin Layer Chromatography is a chromatographic technique that is useful for separating organic compounds. Because of the simplicity and rapidity of TLC, it is often used to monitor the progress of organic reactions and to check the purity of products. Thin-layer chromatography consists of a stationary phase immobilized on a glass or plastic plate and a solvent. The sample, either liquid or dissolved in a volatile solvent, is deposited as a spot on the stationary phase. The constituents of a sample can be identified by simultaneously running standards with the unknown. One edge of the plate is then placed in a solvent reservoir and the solvent moves up the plate by capillary action. When the solvent front reaches the other edge of the stationary phase, the plate is removed from the solvent reservoir. The separated spots are visualized with ultraviolet light or by placing the plate in iodine vapor. The different components in the mixture move up the plate at different rates due to differences in their portioning behavior between the mobile liquid phase and the stationary phase.

2. Gas chromatography (GC)

Gas chromatography is a chromatographic technique that can be used to separate volatile organic compounds. A gas chromatograph consists of a flow mobile phase, an injection port, a separation column containing the stationary phase, and a detector. The organic compounds are separated due to differences in their partitioning behavior between the mobile gas phase and the stationary phase in the column.

Mobile phases are generally inert gases such as helium, argon, or nitrogen. The injection port consists of a rubber septum through which a syringe needle is inserted to inject the sample. The injection port is maintained at a higher temperature than the boiling point of the least volatile component in the sample mixture. Since the partitioning behavior is dependent on temperature, the separation column is usually contained in a thermostat-controlled oven. Separating components with a wide range of boiling points is accomplished by starting at a low oven temperature and increasing the temperature over time to elute the high-boiling point components. Most columns contain a liquid stationary phase on a solid support. Separation of low-molecular weight gases is accomplished with solid adsorbents.

After the components of a mixture are separated using gas chromatography, they must be detected as they exit the GC column. The thermal-conductivity (TCD) and flame-ionization (FID) detectors are the two most common detectors on commercial gas chromatographs. The TCD is not as sensitive as other detectors but it is non-specific and non-destructive. The FID is extremely sensitive with a large dynamic range; its only disadvantage is that it destroys the sample. The requirement of a GC detector depends on the separation application.

3. Gas chromatography/Mass spectrometry (GC/MS)

Mass spectrometers use the difference in mass-to-charge ratio (m/e) of ionized atoms or molecules to separate them from each other. Mass spectrometry is therefore useful for quantitation of atoms or molecules and also for determining chemical and structural information about molecules. Molecules have distinctive fragmentation patterns that provide structural information to identify structural components. The general operation of a mass spectrometer is:

1. create gas-phase ions
2. separate the ions in space or time based on their mass-to-charge ratio
3. measure the quantity of ions of each mass-to-charge ratio

The ion separation power of a mass spectrometer is described by the resolution, which is defined as R = m/ delta m, where m is the ion mass and delta m is the difference in mass between two resolvable peaks in a mass spectrum. e.g., a mass spectrometer with a resolution of 1000 can resolve an ion with a m/e of 100.0 from an ion with a m/e of 100.1.

In general a mass spectrometer consists of an ion source, a mass-selective analyzer, and an ion detector. The magnetic-sector, quadrupole, and time-of-flight designs also require extraction and acceleration ion optics to transfer ions from the source region into the mass analyzer.

Capillary gas chromatography is usually coupled with mass spectrometry and infrared spectrometry. The separated components of the mixture enter the spectrometer one by one, so that each of them can be analyses separately.

In gas- phase infrared spectrometry, a beam of infrared light is directed at the vaporized sample and the absorption of the light is measured the degree of absorption depends on the nature of the compound analyses and the wavelength of the light. The analysis results in an absorption spectrum that is unique to the compound tested. The identity of compound is established by comparison with spectra of reference compounds.

In the mass spectrometer the compound to be analyses is bombarded with high-energy electrons. This results in a partial break-up of molecule and the fragments. The mass and electric charges of the resulting ions are recorded and the identity of the original molecule can be established by comparing the information with reference data stored in a computer. If the identity of the compound cannot be established directly from the reference data, it can be inferred by piecing together the various fragments.

4. NMR spectrometry

More detailed information on compound is obtained by NMR spectrometry. In the resonance spectrum specific peaks represent all different H bonds. To identify the compounds tested; this pattern of peaks can be compared with reference data. If no matching reference data are available, the information combined with the results of mass spectrometer analysis can be used to infer the chemical nature of the compound.

Quality standards [18, 31, 33]

The most important systems of standards for essential oils are those of the International Organization for Standardization (ISO), the Essential Oil Association of the United States (EOA) and the International Fragrance Association (IFRA).

The ISO type defines protocols and methods of analysis to be used in the establishment of particular parameters as follow.

- Sampling - ISO 212:1973
- Preparation of test samples - ISO 356:1996
- Determination of relative density at 20°C -- Reference method - ISO 279:1998
- Determination of refractive index - ISO 280:1998
- Determination of optical rotation - ISO 592:1998
- Evaluation of miscibility in ethanol - ISO 875:1999
- Oil of lime [*Citrus aurantifolia* (Christm.) Swingle], obtained by distillation - ISO 3519:1997
- Oil of lime (cold pressed), Mexican type [*Citrus aurantifolia* (Christm.) Swingle], obtained by mechanical means - ISO 3809:2004
- Gas Chromatography (GC) on capillary columns - ISO 7609:1985
- High Performance Liquid Chromatography (HPLC) - ISO 8432:1987

2.2 Lime Essential Oil [14, 34, 35]

Lime

Scientific name:	*Citrus Aurantifolia* Swingle
Synonyms:	Common lime, Key Lime, Persian Lime, Mexican Lime, Sour lime, West Indian Lime, *C.Medica Var. Acida, C. Latifolia*
Family:	Rutaceae
Origin:	Asia and India. Now it cultivates in Mexico, Peru, Brazil, Italy, the West Indies and the Americas

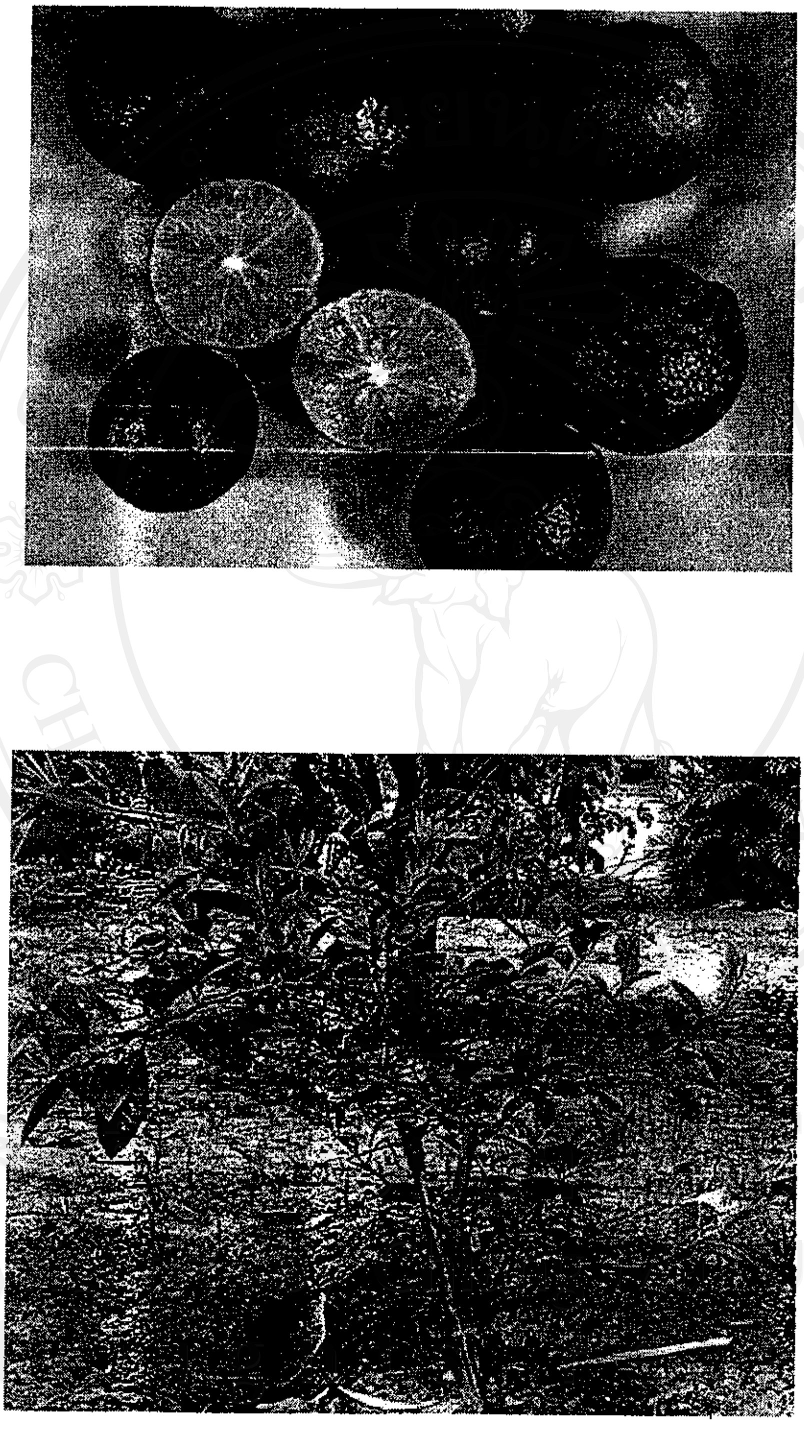


Figure 2.9 Fruit and tree of lime called Manao Namhom

Botanical characteristics:

Lime is an evergreen tree growing up to 4.5 meters (15 feet), with smooth, green leaves, stiff sharp spines and small, white flowers. The fruit is green and although there are many varieties of limes, they usually measure about two inches in diameter.

Typical: In Thailand, there are many varieties of limes, e.g. Namhom, Pan, Dankwien, Tawai, Seeque etc.

Scent: Uplift and re-energize the spirit.

Extraction: Cold expression and Steam distillation

Distilled lime oil is obtained by steam distillation of the crushed or comminuted peels. It is a pale yellow or almost water-white mobile liquid of sharp, fresh, terpene-like, somewhat perfumery-fruity citrus-type odor. The oil is very volatile, but shows increasing sweetness on drying out.

Expressed lime oil is the oil that is expressed from the peel of unripe, green limes. It is a yellowish green, olive-green or dark green mobile liquid of intensely fresh, rich and sweet, peel-like odor. In perfumes, the expressed lime oil is often preferred since the terpency note of the distilled oil is a note rarely wanted in perfumery.

Mixes Well With:

basil, bergamot, citronella, clary sage, eucalyptus, geranium, sage, ginger, grapefruit, hyssop, jasmine, juniper, lavender, sandalwood, neroli, nutmeg, rosemary, rosewood, tangerine, and ylang ylang

Pharmacological Actions:

antirheumatic, antiscorbutic, antiseptic, antiviral, aperitif, tonic, bactericidal, febrifuge, restorative

Traditional Use:

soothing agent, astringent, skin conditioner; a remedy for indigestion, heartburn, nausea, fevers, coughs and various respiratory disorders.

Physical and chemical properties:

Table 2.2 Physical and chemical properties of lemon oil and lime oil [36] .

Property	Lemon Oil	Lime Oil
Color[1]	pale to deep yellow or greenish yellow	colorless to greenish yellow
Odor[1]	fresh citrus, intense	fresh citrus, intense
Specific Gravity[1]	0.849-0.855	0.855-0.863
Refractive Index[1]	1.4740-1.4670	1. 4745-1.4770
Optical Rotation[1]	+57° to +65.6°	+34° to +47°
Stability[2]	air/light sensitive	air/light sensitive
Solubility		
Water[1]	insol.	insol.
Ethanol[1]	sol.	sol.
Propylene glycol[1]	sol.	sol.

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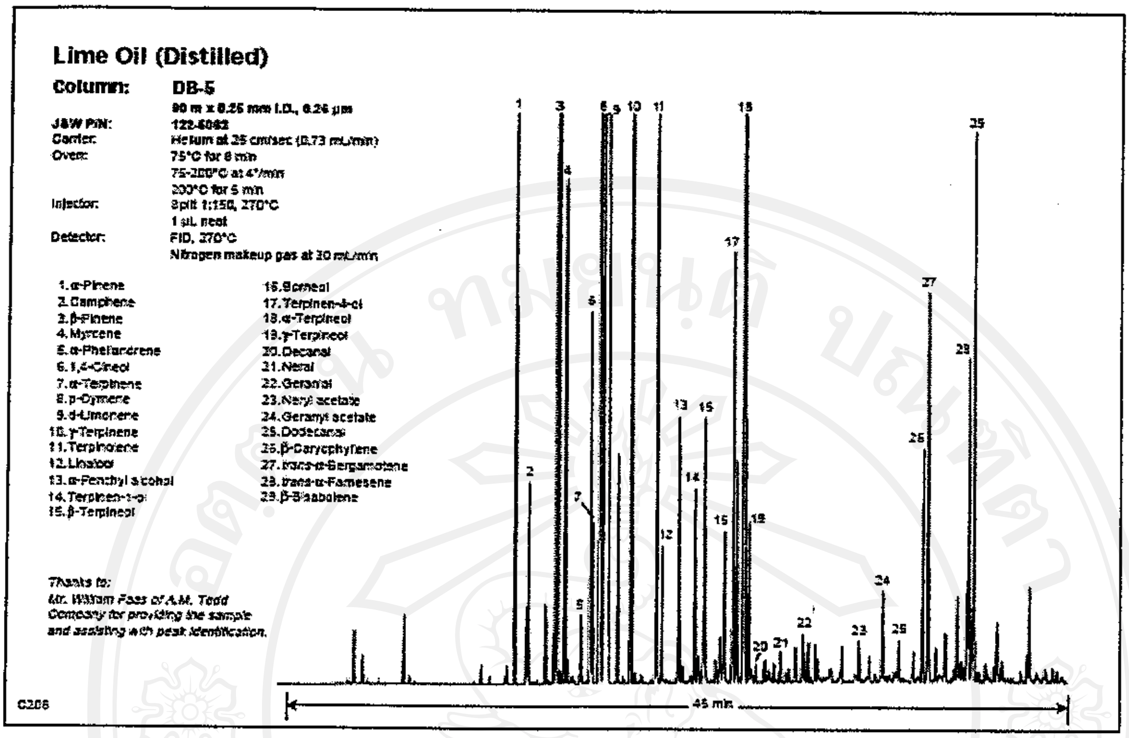
[1]Secondini, 1990, [2] Poucher, 1991

Chemical components:

α-pinene, camphene, β-pinene, myrcene, *p*-cymene, limonene, γ-terpinene, terpinolene, linalool, terpinen-4-ol, α-terpineol, neral, geraniol, neryl acetate, geranyl acetate, caryophyllene, *trans*-α-bergamotene, β-bisabolene



Figere 2.10 chemical components of distilled lime oil by selected ion monitoring GC/MS [37].

Aromatherpy – Home use

Skin Care: acne, anemia, brittle nails, boils, chilblains, corns, cuts, greasy skin, herpes, insect bites, mouth ulcers, spots, varicose veins, and warts.

Circulation, Muscles and Joints:

arthritis, cellulites, high blood pressure, poor circulation, nosebleeds, obesity (congestion), rheumatism.

Respiratory System:

asthma, throat infections, bronchitis, catarrh

Digestive System: dyspepsia.

Immune System: colds, flu, fever and infections.

Clinical potential:

There is hardly any verifiable medicinal data on the whole oil. There is a significant amount of research on its main component d-limonene, however these pieces of research are of little relevance to its use in products. It is used because of its wonderful fragrance, which can have profound psychological effects.

Particularly use for treating people who are 'down in the dumps'. Lime oil can make an absolutely wonderful refreshing and mentally stimulating fragrance. It is particularly suitable for essential oil diffusers.

Safety data:

- Lime oil tested at 15% caused no irritation or sensitisation on humans. Kligman A. 1971, Report to the R.I.F.M.
- Distilled Lime oil showed no photosensitising effects, but expressed Lime oil was found to be a strong photosensitiser. Urbach F. et al. 1972. Report to the R.I.F.M.
- The acute ORAL LD50 in rats exceeded 5 g/kg. The DERMAL LD50 in rabbits also exceeded 5 g/kg. Hart 1971. Report to the R.I.F.M.
- Lime oil was given GRAS status by FEMA in 1965, and was approved by the F.D.A. for food use. The Council of Europe (1970) allowed its use in food with the possible limitation of the active principle. FEMA No. 2631.

Contra indications:

There are no known scientific reasons why this oil should cause any problems during pregnancy. Ensure lime oil does not come into contact with the eyes. Keep all essential oils well away from children.

Both Lime and Lemon oils have a very uplifting fragrance and give a woman a sense of well being when massaged. Lime oil refreshes a woman mentally and is a very stimulating fragrance.

Instructions for use:

To be used throughout pregnancy and labor for massage. The lemon and lime oils used in the product are uplifting, so will give women a boost. The oils used are invigorating.

2.3 Fermentation [38,39]

Fermentation is chemical transformation of organic substances into simpler compounds by the actions of enzymes, complex organic catalysts produced by microorganisms. Originally, it referred to the enzymatic breakdown of carbohydrates in the absent air. It is also used much more broadly to refer to the bulk growth of microorganisms on a growth medium. No distinction is made between aerobic and anaerobic metabolism when the word is used in this sense

The prior fermentation typically refers to the fermentation of sugar to alcohol or lactic acid using yeast. In industrial practice it refers to any process by which are transformed raw materials by the controlled action of carefully selected strains of microorganisms into definite products.

Since fruits ferment naturally, fermentation precedes human history. However, humans began to take control of the fermentation process at some point. There is strong evidence that people were fermenting beverages in Babylon circa 5000 BCE, ancient Egypt circa 3000 BCE, pre-Hispanic Mexico circa 2000 BCE, and Sudan circa 1500 BCE. There is also evidence of leavened bread in ancient Egypt circa 1500 BCE and of milk fermentation in Babylon circa 3000 BCE. The Chinese were probably the first to develop vegetable fermentation.

French chemist Louis Pasteur was the first zymologist, when in 1857 he connected yeast to fermentation (formerly called zymnosis). Pasteur originally defined fermentation as respiration without air. The German Eduard Bucher, winner of the 1907 Nobel Prize in chemistry, later determined that fermentation was actually caused by a yeast secretion that he termed zymase. W.Kuhne gave the name enzymes. The research efforts undertaken by the Danish Carlsberg scientists greatly accelerated the gain of knowledge about yeast and brewing. Now fermentation is included in biotechnology that collected the various acknowledgements: biochemistry, microbiology, genetic engineering and recombinant DNA for developing advance technology in industrial scale.

The primary benefit of fermentation is the conversion, e.g. converting juice into wine, grains into beer, and carbohydrates into carbon dioxide to leaven bread. This process is often used to produce or preserve food.

According to Steinkraus (1995), traditionally food fermentation serves five main purposes:

1. Enrichment of the diet through development of a diversity of flavors, aromas, and textures in food substrates.
2. Preservation of substantial amounts of food through lactic acid, alcoholic, acetic acid, and alkaline fermentation.
3. Enrichment of food substrates biologically with protein, essential amino acids, essential fatty acids, and vitamins.
4. Detoxification during food fermentation processing.
5. A decrease in cooking times and fuel requirements.

Fermentation has some benefits exclusive to foods. Fermentation can produce important nutrients or eliminate anti nutrients. Food can be preserved by fermentation, since fermentation uses up food energy and can make conditions unsuitable for undesirable microorganisms. For example, in picking the acid produced by the dominant bacteria inhibit the growth of all other microorganisms' pathogenic and putrefactive bacteria.

In ancient products of fermentation are fermented foods, beverages, organic acids and organic solvents. Later, demand of fermented products is changed. The interest products are antibiotics. At present, scientists use microbiology and genetic engineering to develop many new products such as Table 2.3.

Table 2.3 Fermented products for some uses

Products	Samples
Foods	Cheese, bread, fermented milk, vinegar.
Beverages	Wine, beer, brandy, distilled.
Organic acids	Lactic acid, acetic acid, citric acid, gluconic acid, fumaric acid.
Organic solvents	Ethanol, butanol, acetone.
Antibiotics	Penicillins, steptomycin, tetracyclin, neomycin, kanamycin.
Enzymes	Amylase, asparaginase, catalase, lipase, cellulase, invetase.
Vitamins and growth stimuants	Riboflavin, cyanocobalamin, ascorbic acid, gibberelin.
Amino acids	Glutamic acid, lysine, leucine, valine.
Flavors and fragrances	Terpenes, vanillin, benzaldehyde.
Flavor enchancers	Monosodium glutamate, nucleotides.
Nucleotides	Inosinic acid, xanthine monophosphate, guanosine monophosphate.
Bioelectronics	Biosensors, biochips.
Hormones	Insulin, interferon.

2.4 Biotechnological Production of Flavors and Fragrances [2, 40, 41]

Most aroma compounds are now produced via chemical synthesis or extraction. The increased customer demand for natural flavors and fragrances has pushed attempts to produce them by biotechnological processes, i.e. biotransformation / bioconversion and fermentation / de novo synthesis. Microbial processes have traditionally played an integral role in development of complex mixtures of flavor and aroma chemicals since the discovery of beer, wine, cheese and soy sauce thousands of years ago. The reviews of the microbial aroma generation indicate that fungi are important sources for flavoring compounds because of their relatively rapid growth, the variety of aroma mixture, the utilization of waste substrates and the potential of solid state fermentation. In the past few years, microbial biocatalysis has also begun to use to increase the commercial production of many flavor and fragrance aroma chemicals. Today microbiological techniques are more applied to enhance the efficiency of many microbial biocatalysis for the production of specific flavor and fragrance chemicals.

These prior biotechnological aroma productions use cultivation media with adding product precursors to synthesis of one or several aroma substances. While de novo synthesis uses the whole metabolic spectrum of the microorganisms to produce a mixture of several aroma compounds, the biotransformation / bioconversion leads to only one major product, produced by single (biotransformation) or several (bioconversion) bio-chemical steps. Biotechnological aroma production often lack economic profitability, mostly due to low productivities. Because of inhibitory effects of the microorganisms is strong even at low concentration. In situ product removal (ISPR) is a powerful tool to overcome these limitations. So the further developed process, i.e. integrated bioprocess (IBP) can use to increased production of natural flavors and fragrances. Their possible production by biotransformation, bioconversion and de novo synthesis using microorganisms, plant cells or isolated enzymes is shown as follow.

Laseter *et al.* [42] studied volatile terpeniods from aeciospores of *Cronartium fusiforme* by GLC and GLC-MS. It was present that several aromatic compounds were found. The major monoterpenoid hydrocarbons were α-pinene, β-pinene, δ-carene, myrcene, limonene, β-phellandrene, and δ-terpinene. A number of monoterpenoid alcohols were also present of which terpen-4-ol predominated. Some acyclic sesquiterpenes are β-farnesene and β-citronellol.

Lanza and Palmer [43] studied the biosynthesis of monoterpenes by *Ceratocystis monilliformis*. It was shown that the aroma compounds were geraniol, nerol, citronelol, linalol, α-terpineol, geranial and neral by GC-MS identification. The pathway for this production was formed via the mevalonate pathway, which has been the first information on the biosynthesis pathway for free monoterpenes in a microbial system.

Hanssen [44] studied the sesquiterpene alchohols from *Lentinus lepideus* on liquid cultures. It was found that several sesquiterpene alchohols were mainly 4, 10-dimethy-7-isopropyldecaline skeleton (candinols, muurolols, cubenols). Besides these, *trans*, *trans*-farneol and the bicyclofarnesol drimenol have been found too.

Yamauchi *et al.* [45] studied production and application of a fruity odor in a solid-state culture of *Neurospora sp.* ATCC 46892 using pregelatinized polished rice. It was found that the main components of the aroma were ethyl caproate, ethanol and isoamyl alcohol by GC analysis. The cell-free extract of the solid state culture (which call N-koji) contained a strong alcohol acyltransferase activity, which caused abundant ethyl caproate production in the culture. The odoriferous N-koji was applied to sake, a Japanese alcohol beverage, and as a result as sake rich in ethyl caproate was obtained. The solid state culture was one of the useful methods for fruity odor production.

Tan *et al.* [46] emphasized to study the optimization of the process of bioconversion of (R)-(+)-limonene by *Penicillium digitatum* (NRRL 1202). It was found that bioconversion activity increased more than 4 fold after substrate induction. Aeration was critical to this bioconversion. The optimum conditions for bioconversion were 1.6 mg/ml dissolved oxygen (D.O.), 1 % (v/v) limonene, pH 4.5, temperature 28°C and 1-day biotransformation. Bioconversion only found in the early to mid-log phase of growth. The highest a-terpineol yield, 3.2 mg/ml, was obtained by a sequential substrate induction. Racemic limonene was enantio-selectively and enantio-specifically converted to (4R)-(+)-α-terpineol by *P. digitatum* mycelia.

Demyttenaere *et al.* [47] studied biotransformation of (R)-(+)-and (S)-(-)-limonene by fungi and the use of solid phase micro extraction for screening. It was presented that of (+)-and (-)-limonene was converted by *Penicillium digitatum* to α-terpineol (main metabolite), *cis*- and *trans-p*-menth-2-en-1-ol, neodihydrocarveol and limonene oxide (minor metabolite) using liquid cultures. The bioconversion of (R)-(+)-and (S)-(-)-limonene by *Corynespora cassiicola* yielded (1S,2S,4R)- and (1R,2R,4S)-limonene-1,2-diol respectively. The optimum conversion of limonene to α-terpineol by *Penicillium digitatum* was obtained after 8 hours (yield up 100%).

Bluemke and Schrader [48] studied the integrated bioprocess (IBP) for enhanced production of natural flavors and fragrances by *Ceratocystis monilliformis.* It was presented that the main products of the fungus were ethyl acetate, propyl acetate, isobutyl acetate, isoamyl acetate, citronellol and geraniol. Total yield of aroma compounds produced is higher in IBP compared with batch cultivation. In addition, permeates obtained from pervaporation consist of highly enriched mixtures of produced flavors and fragrances. This process can use to solve problem in low productivities or poor concentrations of the target compounds in the fermentation broth. Because this process used situ product removal to remove inhibition product to increased microbial growth rates and higher biomass activity that leads to increased aroma production.

Carrau *et al.* [49] studied the de novo of monoterpenes by *Saccharomyces cerevisiae* wine yeasts. It was presented that biosynthesis of monoterpenes by *S. cerevisiae* in the absence of grape derived precursors was shown here to be of de novo origin in wine yeast strains. Linalool and α-terpineol were the terpenes produced in greatest abundunce by *S. cerevisiae* all wine yeasts tested. The Higher concentration of assimilable nitrogen increased accumulation of linalool and citronellol. The amount of linalool produced by some strains of *S. cerevisiae* could be of sensory importance in wine production.

Peinado *et al.* [50] studied uses of a novel immobilization yeast system for winemaking. It was found that *Penicillium* was used to immobilize *Saccharomyces* *cerevisiae*, without using physico-chemical external supports, to form yeast biocapsules. The biocapsules, once the *Penicillium* was killed by the ethanol produced, were used in grape must fermentation. Acetadehyde, isobutanol, L-proline and aspartic acid in final wine were higher with the biocapsules than with free yeast cells. The biocapsules can use to protect microorganisms from inhibition product to increase volatile aroma wine.

2.5 Bio-Fermented Products (BFP) [6]

Bio-fermented products are the brown liquids extracted from the finished fermentation of various plants with and without using probiotic microorganisms as a starter in the appropriate circumstance. They have sour taste and smell. The pH values of them are about 3-4.

2.5.1 Production of BFP

1. Natural fermentation

This process uses indigenous microorganisms in nature to roll on fermentation. It is easy to produce, be low cost and energy, but cannot control type and counts of microorganisms those effects on fermentation process.

The materials are fermented in close system vessel for 3 months in formula as follow.

Plant materials : brown sugar cane : water = 3 : 1 : 10 by weight

2. Starter fermentation

2.1 Using the old BFP, this contents probiotics and prebiotics

It is easy to produce, be low cost and energy, but cannot control type and counts of microorganisms those effects on fermentation process.

The materials are fermented in close system vessel for 2 months in formula as follow.

Plant materials : old BFP : brown sugar cane : water = 3 : 1 : 1 : 10 by weight

2.2 Using the inoculated microorganisms

It uses culture media to increase counts and type of microorganisms for control quality and yield content but it is higher cost and energy. The selective Probiotics, *lactobacillus* spp., is popular to use as starter for fermentation.

The materials are fermented in close system vessel for appropriated time in formula as follow and add starter solution.

Plant materials : brown sugar cane : water = 3 : 1 : 10 by weight + Starter

3. Industrial fermentation

This scale up process uses knowledge in biochem engineering to design fermenter or big bioreactor with modern electronic system or computer for control the appropriate factors on microbial culture. This method can control volume productivity of target products. Cost and energy are too high.

2.5.2 Biological properties and antioxidative capacity of BFP

The recent year many researches were studied their properties and production to show that the observed microorganisms were lactic acid bacteria; coli form bacteria, yeast and mold. But some BFP had no any observed microorganisms, it may be up to materials and production processes and also the pH that lower than 4 of BFP that could inhibited the growth of the pathogens. The pathogenic bacteria, *Bacillus cereus, Clostridium perfringens, Samonella* spp., *Staphylococus aureus* and *Escherichia coli,* were not found in these products. BFP had the antimicrobial activities against the pathogenics: *Bacillus cereus, Escherichia coli, Pseudomona aeruginosa, Candida albicans, Samonella* spp., *Shigella* spp., *Staphylococus aureus* and *Vibrio parahaemolyticus.* The researches were studied microbial test, their properties and production formula as follow.

Nongnit *et al.* [7] collected Bio-fermented products from Ratchathani Asoke community to study physical properties and microbiological test. It was found that the pH values of these products were about 3-4. BFP had no surfactant property. The pathogenic bacteria, *Coliform, Samonella* spp., *Streptococcus* spp., *Staphylococus aureus, Pseudomona aeruginosa, Escherichia coli;* yeast and mold were not found in BFP. BFP had the antimicrobial activities against the pathogenics: *Staphylococus aureus* and *Escherichia coli* with minimum inhibitory concentration (MIC) 3.1%. So Shampoo or other products contained with BFP no need to use preservatives. It was presented that shampoo contained with BFP had no any observed microorganisms, not severely irritated eye, skin and not destroyed hair.

Warunee [8] studied the production process of Bio-fermented products. It was found that the popular formula was brown sugar: fruit: water = 1: 3: 10 by addition of water after mixed fruit and sugar 15 days then fermented 3 months before filtered. The pH values of all products were less 4. Mold and Lactic acid bacteria were not found in BFP. The old BFP had less yeast than the new. The colony size of yeast of BFP containing honey was very small.

Suchada *et al.* [9] have investigated in microbiological test and contaminated substance of BFP containing *Morinda citrifolia* Linn. It was presented that yeast, mold and the pathogenic, *Escherichia coli, Clostridium perfringens, Samonella* spp., *Staphylococus aureus* and *Escherichia coli,* were not found in BFP. MPN Coli forms were found less 1.1. Synthetic color and preservative (benzoic acid) were not found. Nutrients were carbohydrate, calcium, sodium, vitamin B1 and B2. The pH values of the 3-month BFP were about 3 and alcohol was too low 0.08%.

Sasithon [10] investigated in chemical and biological properties of fermented medicinal plant juices (FMPJ) containing *Morinda citrifolia* Linn. It was presented that the pH values of final products were 3.2-4.4. The main products were lactic acid, acetic acid and ethanol 0.02-1.93% (v/v). Methanol, acetaldehyde and iso-propanol were only detected at first period of fermentation (day 0-6). The observed microorganisms were lactic acid bacteria; coli form bacteria, yeast and mold. The pathogenics, *Escherichia coli, Staphylococus aureus, Bacillus cereus, Clostridium perfringens, Samonella* spp. and *Shigella* spp., were not found in all samples. FMPJ had the antimicrobial activities against the pathogenics: *Escherichia coli, Staphylococus aureus, Pseudomona aeruginosa, Bacillus cereus* and *Candida albicans.* All values of kinetics parameters of formulas that fermented with starter culture were higher than the formulas that fermented without starter culture.

Besides some researches were studied increased antioxidative capacities of BFP after fermentation. Biologically fermented plant juices (BFPJ) were superior to medicinal plant juices in antioxidative capacity. Accordingly BFPJ might be potential antioxidants for application as neutraceutical products. Scientific data obtained will be used as a guide for quality standard, further information on fermentation process, preliminary efficacy. The results lead to the further development of these products as a neutraceutical, agricultural, environmental and household products.

Chaiwat *et al.* [11] have investigated in antioxidative capacity of biologically fermented plant juices (BFPJ) containing *Morinda citrifolia* Linn with different production processes. Antioxidative capacity of these products was evaluated by ABTS free radical decolorization assay, ferric reducing ability power (FRAP) assay and chelating effect on ferrous assay. BFPJ have increased antioxidative capacity after fermentation. It was shown that the highest antioxidative capacity were found on the 15th day of fermentation process and then rapidly decreased until the 20th day of fermentation process. The antioxidative capacity increased again at 90th days of fermentation process and maintains at higher level than the non-fermentation (day 0). The formulas, which using the *L. casei* as a starter had no different antioxidative capacity when compared to the formulas which did not use the *L. casei* as a starter. The pasteurized formula had antioxidative capacity less than the formula that used KMS. It was presented that the formula that used brown sugar and KMS had the highest antioxidative capacity.

Sartjin [12] studied antioxidative capacity of biologically fermented plant juices (BFPJ) containing *Phyllanthus emblica* Linn with different production processes. Antioxidative capacity of these products was evaluated by ABTS free radical decolorization assay, ferric reducing ability power (FRAP) assay and chelating effect on ferrous assay. It was shown that the highest antioxidative capacity were found on the 15th day of fermentation process and then rapidly decreased until the 20th day of fermentation process. The antioxidative capacity increased again at 120th days of fermentation process and maintains at higher level than the non-fermentation (day 0). The formulas which using the *L. casei* as a starter had no different antioxidative capacity compared to the formulas which did not using the *L. casei* as a starter. The formula that used brown sugar had antioxidative capacity by ABTS higher than the formula that used honey or white fine sugar. The formula that used honey had antioxidative capacity by FRAP higher than the formula that used brown sugar.

The potential antioxidants of BFP are interesting to use in beverage, medical cosmetics and household products. Due to the intense sour smell of them is not satisfied for uses. Perfume oils and fragrances are used to cover this odor. But it is generally known that the synthetic oils are not safety to use in our product [14]. The new application of BFP may be use to produce nature flavors and fragrances as follow.

1. Fermentation with the odorous plant

By using the odorous plant, brown sugar and water in formula 3: 1: 10 (w/w/w) and ferment for 3 months in close system vessel. The final odor is change because of much change in essential oil during fermentation.

2. Fermentation with the old BFP

While the odorous plant is preserved with BFP, it can use to increase aroma in BFP during fermentation. For avoidance many changes in essential oil during fermentation, it is only used BFP without sugar and water added in this process by using BFP: plant in formula 1:1 (w/w) in close system vessel. The acidic fermentation is taken for the appropriate time, after that essential oil is gentle released to BFP without use of organic solvents and heat. So this new technique can produce natural flavors and fragrances.