Volatile compound analysis

Analyzed using GC-MS system, equipped with :

1. **30 m x 0.25 mm i.d. x 0.25 µm film thickness,**
2. **DB-5ms capillary column.**
3. **The carrier gas was helium at flow rate 0.56682 ml/min, and 1 µml of sample (100 ppm concentration) was injected directly**
4. **The injector and detector temperatures were 230°C and 250°C respectively.**
5. **The running methods were splitless mode, pressure: 3 psi, oven temperature: 70 °C then 10°C/min. to 140°C, and then 5°C/min. to 240°C**

1. **Sample concentration : 100 ppm**
2. **Volume injection : 1 µL**

- Compared with WILEY257 and NIST library use a % quality match greater than 85%
- RI calculation based on *n-alkane* standard (C_{10}-C_{20})

Volatile compound analysis

Essential oil

No.	Volatile compound	RI[a]	% of Composition		No.	Volatile compound	RI[a]	% of Composition	
			Fresh	Dry				Fresh	Dry
1.	eucalyptol	1051	0.31	-	14.	alpha,-curcumene	1490	3.16	4.49
2.	undecane	1108	-	0.14	15.	eremophillene	1508	6.89	4.20
3.	1-nonanol	1174	-	0.09	16.	7-epi-alpha-selinene	1537	-	2.59
4.	decanal	1212	7.32	4.47	17.	ledol	1550	5.99	-
5.	decanol	1274	-	3.34	18.	nerolidol	1564	-	3.67
6.	undecanal	1311	0.58	0.57	19.	globulol	1587	0.95	-
7.	n-decanoic acid	1358	0.21	-	20.	caryophyllene oxide	1601	2.04	5.64
8	1-Nonene	1374	-	2.02	21.	cubenol	1640	0.08	-
9.	beta-elemene	1401	0.64	-	22.	eupatoriochromene	1664	21.71	20.94
10.	dodecanal	1417	19.96	18.72	23.	drimenol	1790	4.74	4.34
11.	beta-caryophyllene	1441	11.07	11.40	24.	Hexahydro farnesyl acetone	1842	-	0.60

[a] Retention indices calculated based on *n*-alkane standard (C_{10}-C_{20})

Starkenmann et al, (2008) mentioned the major compounds of this plant are decanal and caryophyllene.

Volatile compound analysis

Plant extract

No.	Volatile compound	RI[a]	% of Composition					
			Petroleum ether		Acetone		Ethanol	
			1st	2nd	1st	2nd	1st	2nd
1.	beta-pinene	981	-	-	8.06	16.55	13.10	-
2.	beta-cis-ocimene	1025	-	-	-	-	1.61	-
3.	3-carene	1037	-	-	-	2.08	-	-
4.	ocimene	1050	-	-	17.90	36.46	26.44	-
5.	decanal	1208	18.43	12.09	7.73	4.64	4.42	-
6.	methyl hydrocinnamate	1278	-	-	-	-	2.57	-
7.	ethyl dihydrocinnamate	1351	-	-	1.77	4.24	2.71	-
8	copaene	1388	-	-	-	8.52	6.00	-
9.	dodecanal	1409	53.12	38.36	27.14	8.06	7.21	11.35
10.	caryophyllene	1435	5.42	8.07	6.26	6.49	7.07	11.01
11.	cyclododecane	1471	6.13	6.47	-	-	-	-
12.	1,1-diethoxydecane	1472	-	-	8.10	-	-	19.03
13.	germacrene d.	1495	-	-	2.40	3.06	-	-
14.	3,5-di-tert-butylphenol	1505	-	-	5.78	4.81	9.16	10.70
15.	dehydro-cyclolongifolene oxide	1657	8.21	16.48	-	-	-	-
16.	eupatoriochromene	1657	-	-	5.52	-	8.00	19.49
17.	neophytadiene	1834	2.88	5.30	4.71	5.09	6.08	-
18.	ethyl hexadecanoate	1989	5.80	13.24	4.62	-	5.61	28.43

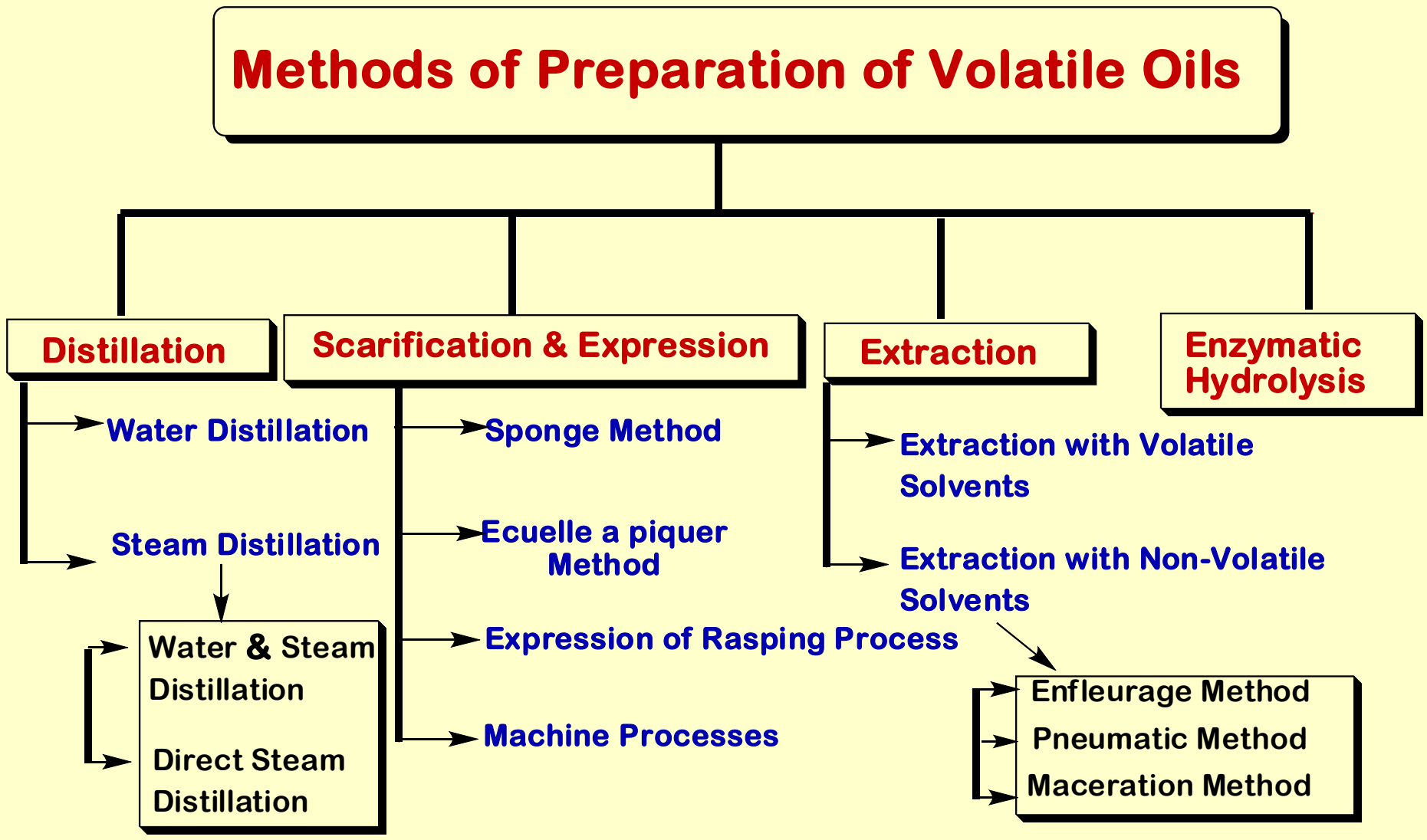
Volatile compound analysis

Analyzed using GC-MS system, equipped with :

1. **30 m x 0.25 mm i.d. x 0.25 µm film thickness,**
2. **DB-5ms capillary column.**
3. **The carrier gas was helium at flow rate 0.56682 ml/min, and 1 µml of sample (100 ppm concentration) was injected directly.**
4. **The injector and detector temperatures were 230°C and 250°C respectively.**
5. **The running methods were splitless mode, pressure: 3 psi, oven temperature: 40 °C then rate 8°C/min. to 100°C, then rate 15°C/min. to 180°C, and the last rate 12°C/min. to 280°C**

1. **Sample concentration : 1000 ppm**
2. **Volume injection : 0.5 µL**

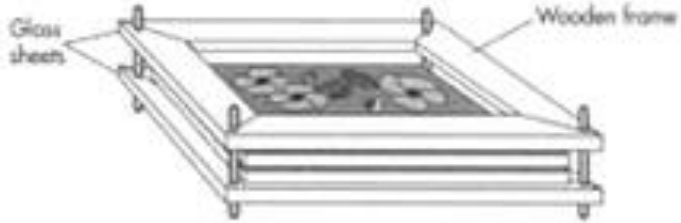
- Compared with WILEY257 and NIST library use a % quality match greater than 85%
- RI calculation based on *n-alkane* standard (C_{10}-C_{20})


Methods of Preparation of Volatile Oils
Distillation
Scarification & Expression
Extraction
Enzymatic Hydrolysis
Water Distillation
Steam Distillation
Water & Steam Distillation
Direct Steam Distillation
Sponge Method
Ecuelle a piquer Method
Expression of Rasping Process
Machine Processes
Extraction with Volatile Solvents
Extraction with Non-Volatile Solvents
Enfleurage Method
Pneumatic Method
Maceration Method

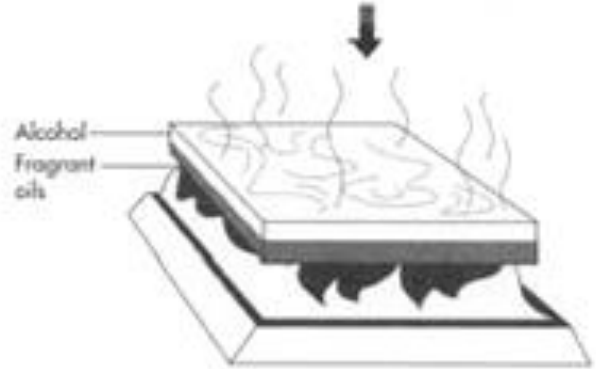
Steam Distillation

Steam passing through plant materials change essential oils to gas and extract.

Enfleurage

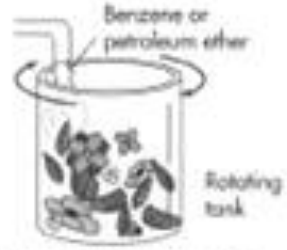


Flowers are spread on glass sheets coated with grease and placed in wooden frames. The flowers are changed by hand until the grease has absorbed enough fragrance.

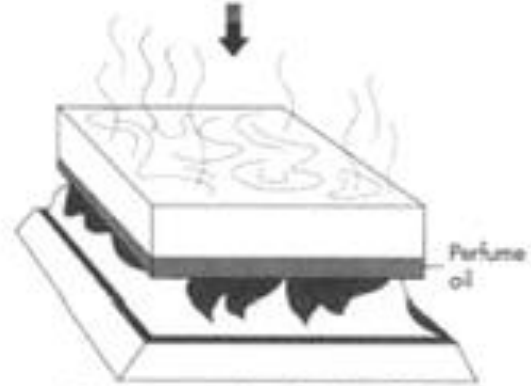


The grease is dissolved in alcohol, which is then evaporated with heat to leave behind the oils.

Solvent Extraction



Flower parts dissolve in the solvents, leaving a waxy material containing the oils.



The waxy material is placed in ethyl alcohol, and the oil dissolves and rises to the top. The alcohol is evaporated and the perfume concentration settles to the bottom.

Expression

Plant materials are pressed to squeeze out oils.

Selection of the suitable method is done according to :

1. The condition of the plant material (moisture content, degree of comminution)
2. The localization of the oil in the plant (superficial or deep)
3. The amount of the oil
4. The nature of the oil constituents

Distillation methods

Principle

- Most volatile oil constituents boil between 150-300°C. In order to reduce decomposition, volatile oils are distilled in the presence of water.

- The mixture will boil below 100°C [Dalton's law of partial pressure : "When 2 immiscible liquids are heated together, they will boil at a temperature below the boiling point of either one"].

- The oil is carried over with steam in the form of vapor

Distillation methods

Application: preparation of thermostable oils, present in large amounts & not rich in esters (e.g. oils of turpentine, peppermint, cardamon, anise, eucalyptus)

Types of distillation:

1. Water-distillation
2. Steam distillation
   - Water-and-steam distillation
   - Direct-steam distillation

Distillation: Terminology

- **Hydrodiffusion** = process by which water or steam penetrates the plant tissues to take over the oil

- **Aromatic water** = **Hydrosols** = distilled aqueous layer saturated with oil e.g. rose, orange flower & peppermint waters

- **Cohobation** = return of aromatic water to the distillation chamber, in water distillation, in order to recover the dissolved oil.

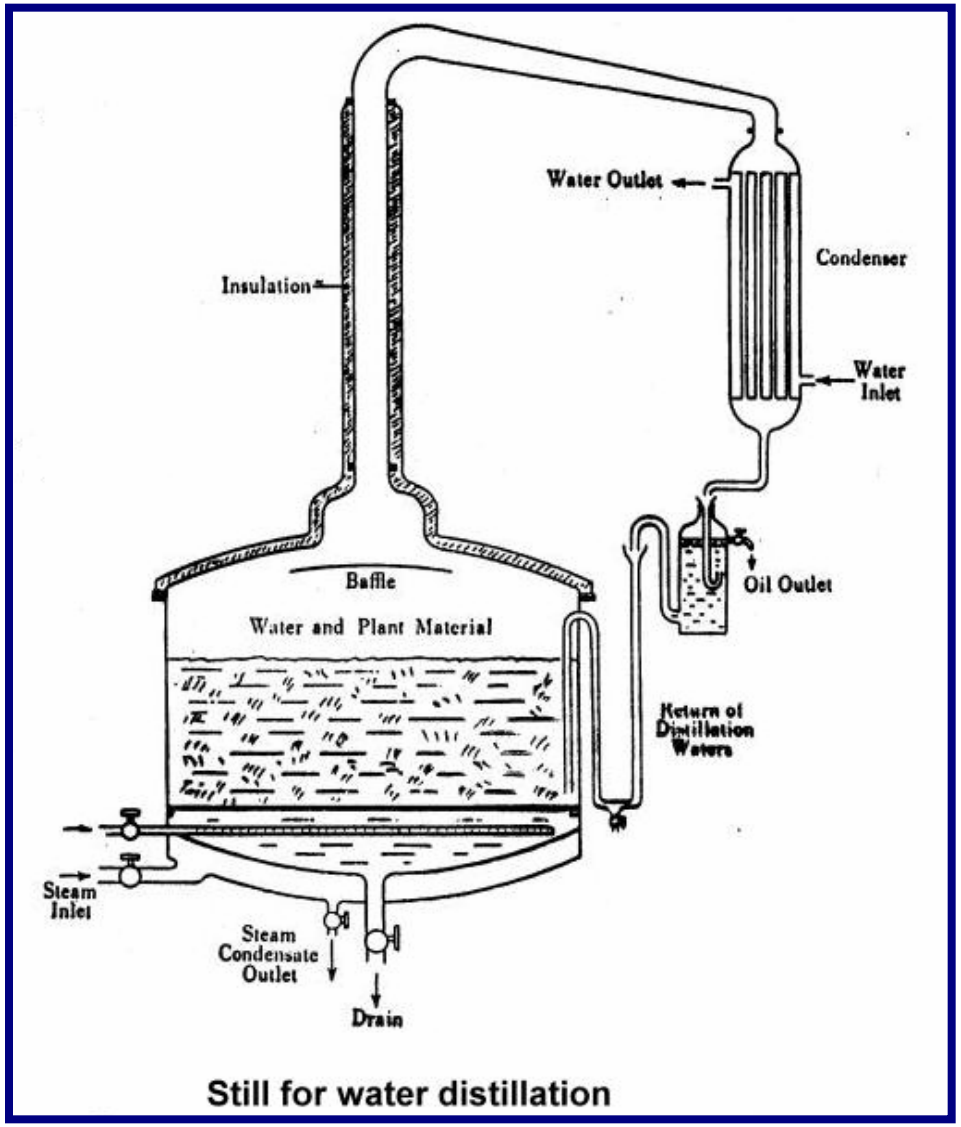
Distillation methods

	H_2O Distillation	Steam Distillation	
		H_2O & Steam	Direct Steam
Plant material	Dried & fresh (petals), not injured by boiling with H_2O	Dried & fresh, injured by direct boiling with H_2O	Fresh (i.e. containing moisture)
Commercial preparations	Oils of turpentine & rose	Oils of clove, cinnamon & citronella	Oil of peppermint
Mode of charging	Plant material dipped in H_2O	-H_2O present but not in contact with the plant. -Steam is generated in the still & penetrates the drug -Dried material is moistened before charging	-H_2O is absent. -Steam is introduced by pipes & forced through the plant material placed on perforated trays
Steam pressure	≈ atmospheric		Can be modified
Temperature	≈ 100°C		Can be modified
Rate & yield	Relatively low	Better	The best
Advantages	-Least expensive -Cohobation is allowed	Hydrolysis is reduced	Method suitable for oils rich in esters & high b. p. constituents
Disadvantages	-Esters are hydrolyzed. -H_2O sol. & high b.p. constituents are not distilled	-Not suitable for powders, efficient if material entire or crushed -Hydrodiffusion may be reduced due to lumping or channeling	

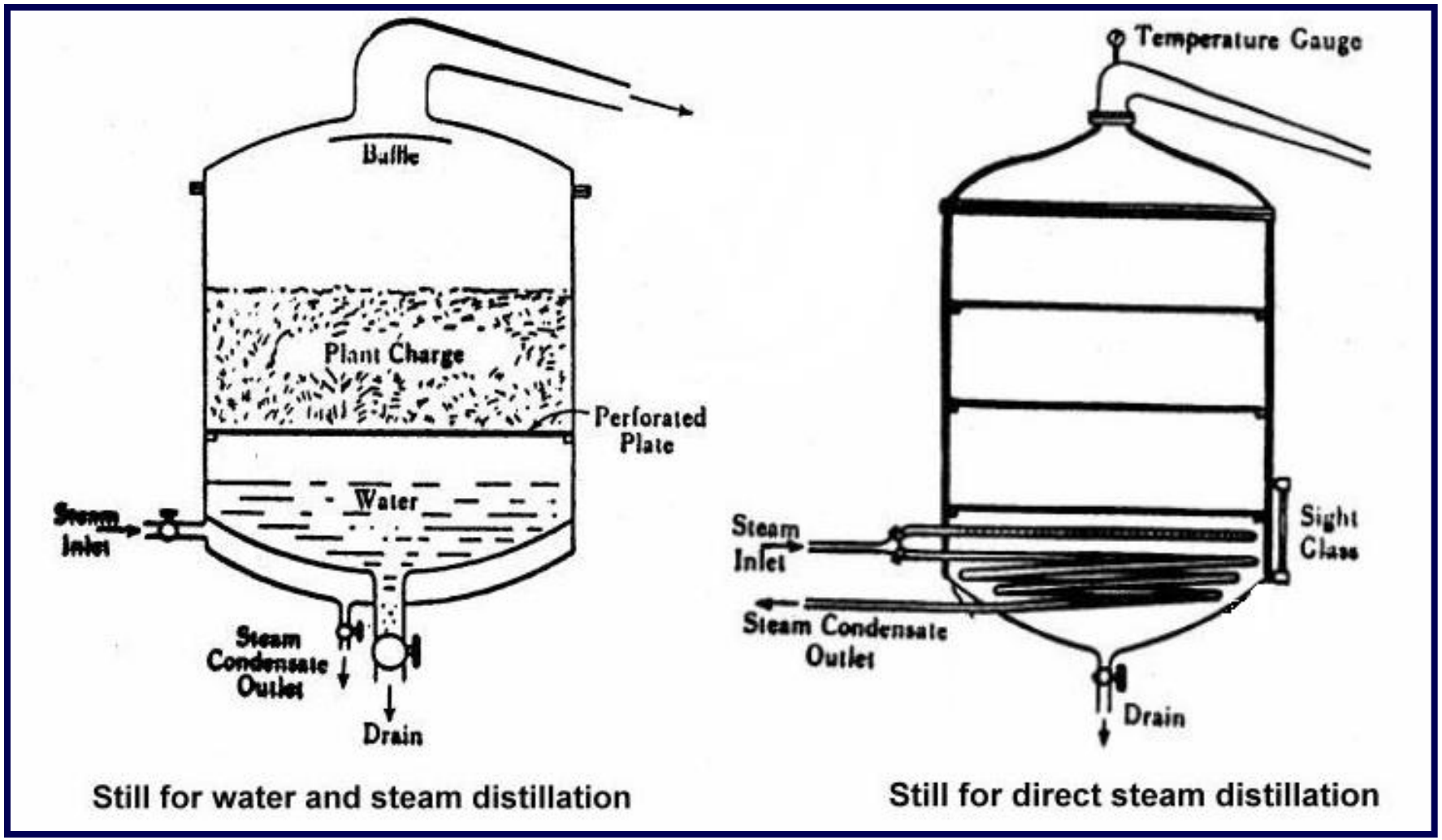
Distillation apparatus

Consists of 3 parts:

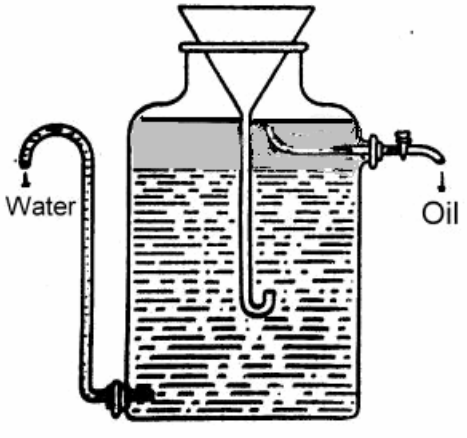
1. The distillation chamber made of stainless steel free from any Fe^{+++} ions to avoid degradation of the oil constituents → darker oils.
2. The condensing system
3. The receiver e.g. Florentine receivers which allow separation of the oily layer from water in the distillate (oils lighter or heavier than water)



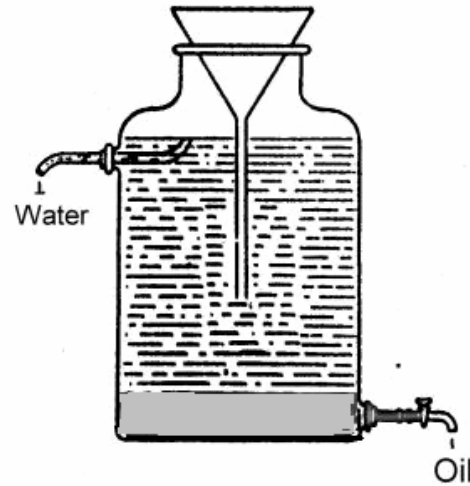
Still for water distillation


Baffle
Plant Charge
Perforated Plate
Water
Steam Inlet
Steam Condensate Outlet
Drain
Still for water and steam distillation
Temperature Gauge
Steam Inlet
Steam Condensate Outlet
Sight Glass
Drain
Still for direct steam distillation

Florentine Receivers



For oils lighter than water



For oils heavier than water

Purification (Rectification) of distilled oils

Bad smelling or dark colored oils are purified by:

1. **Redistillation** or dry distillation under reduced pressure
2. **Dehydration** by passing over anhydrous sodium sulphate

Remarks

1. **Distillation** should be done **just after comminution** [i.e. reduction in size, crushing, powdering) → prevent loss by evaporation or deterioration of the oil.
2. **Coarse comminution** → increase "Hydrodiffusion" → oils with better yield & quality.
3. **High temperature & water** → distilled oils differing in composition from natural oils [artifacts].
4. **Insufficient distillation time** (shorter) → fractionation of the oil.
5. **Hydrolytic products** (e.g. lower alcohols & acids) are water-soluble & remain in the distillation chamber.
6. **Steam volatile impurities** e.g. amines & furfural (degradation product of carbohydrates) contaminate the final product.
7. **Sensitive constituents** could be affected by boiling water e.g.
   - ♣ Esters → hydrolyzed.
   - ♣ Tertiary alcohols → dehydrated → hydrocarbons.
   - ♣ Unsaturated hydrocarbons → polymerized.

Scarification & Expression Methods

Principle

Mechanical procedures carried at **room temperature** & based on **puncturing** & **squeezing** of the plant material to liberate the oil, which is collected.

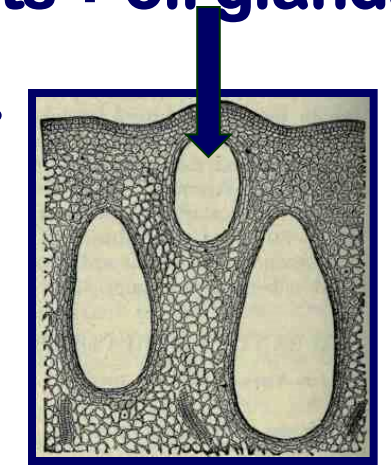
Applications

Preparation of **heat sensitive oils**, **present in large amounts in outer peels of fruits** e.g. ***Citrus*** fruits (Rutaceae) as orange, lemon & bergamot.

Scarification & Expression Methods

The peel of *Citrus* fruits consists of 2 distinct layers:

1. **Outer colored zone** (waxes + pigments + oil glands)
2. **Inner white zone** (pectin + cellulose).



Scarification & Expression Methods

The process involves 3 steps:

1. Squeezing of the peel under a stream of water → emulsion (volatile oil + water + pectin + cellulose + pigments + traces of waxes).
2. Centrifugation (to remove water + pectin + cellulose)
3. Strong cooling (to remove waxes)

Scarification & Expression Methods

A- Sponge Method

Based on squeezing the removed peels e.g. orange

1. Fruits washed, cut into halves & fleshy parts removed.
2. Peels soaked in water, turned inside out then pressed between a convex projection & a sponge.
3. Sponge (saturated with oil emulsion) periodically squeezed in a vessel

The tissue of the sponge serves for:

1. Collection of the oil
2. Filtration of the product from any particles of the inner white zone of the peel.

Solvent extraction methods

Principle

Based on extraction of the volatile oil from the plant material with a suitable solvent

According to the nature of the solvent used, three types are distinguished:

1. **Volatile solvent extraction**
2. **Non-volatile solvent extraction**
3. **Supercritical fluid extraction**

Solvent extraction methods-Application

Preparation of delicate flower oils e.g. jasmine, violet, tuberose & narcissus which are:









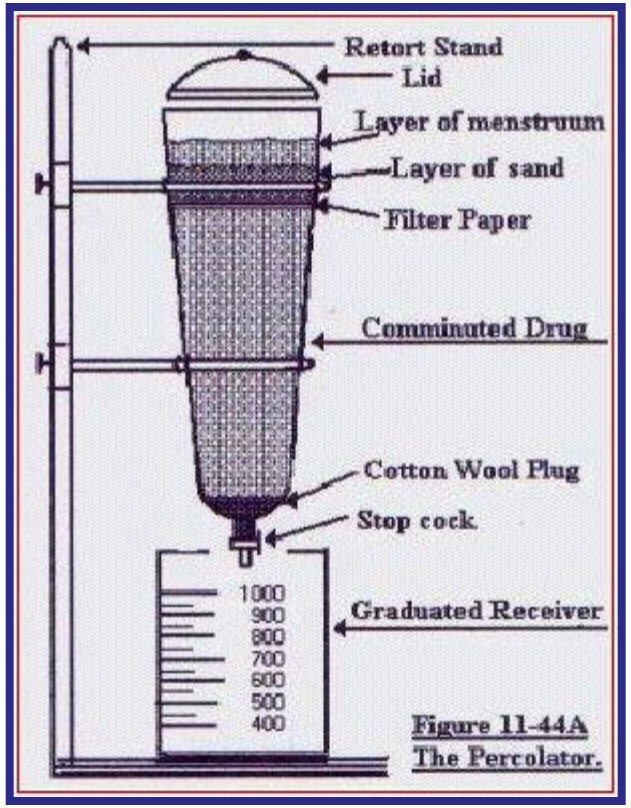
1. Present in very small amounts, not easily obtained by distillation or expression

2. Oils formed of thermolabile constituents (i.e. easily decomposed by heat)

Volatile solvent-extraction

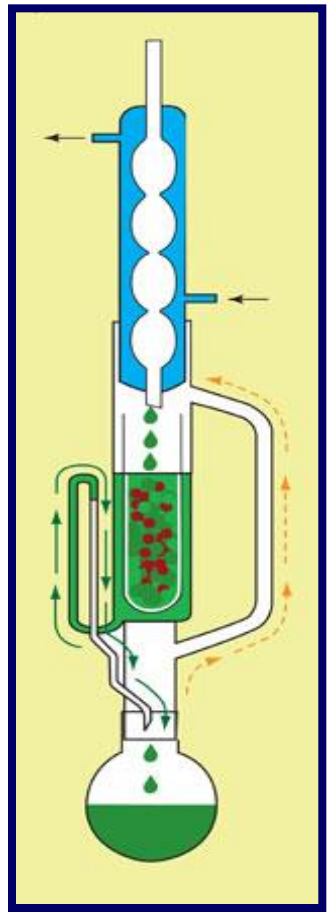
Preparation of "floral concretes"

1. **Solvents used:** petroleum ether & n-hexane
2. **Extraction** ("percolation" or "maceration" at room temperature, "continuous hot extraction" in a Soxhlet apparatus at constant temperature)
3. **Solvent removal** (distillation under reduced pressure)



Percolator

Soxhlet apparatus



Volatile solvent-extraction

Floral concrete = Fragrant constituents + Fats + Waxes + Albuminous matter + Fat soluble pigments

e.g. "floral concrete" of jasmine is semi-solid & yellowish-orange in color.

Floral absolute = consists mostly of the oxygenated constituents of the oil.

- ♣ More expensive & purified than the corresponding concrete.
- ♣ **Preparation:** repeated extraction with absolute alcohol
- ♣ **Impurities:** removed by strong cooling & filtration
- ♣ **Solvent removal** : by distillation.

Non-volatile solvent extraction

Application: Preparation of natural flower oils producing the finest perfumes.

Principle: based on the liposolubility of volatile oils

Solvents:

Lipids of high degree of purity e.g.

- Fats (lard : tallow in a mixture 2:1)
- Fixed (olive oil)

Techniques:

- Enfleurage (hot & cold)
- Pneumatic method
- Maceration (in fixed oils)

Enfleurage Process- Preparation of jasmine oil

♣ Equipment:

Great number of glass plates closely arranged in wooden frames (or chassis).

♣ Procedure:

1. Spread the mixture of fat (lard / tallow 2: 1) on both surfaces of each glass plate.
2. Cover the top of each plate with flowers or petals, so that each layer of flowers is enclosed between 2 layers of fat.
3. Replace old flowers by fresh ones every 2-3 days
4. Repeat the process until the fat is saturated with the oil
5. Remove the last charge of flowers from the fat ("Defleurage")
6. Scrap & collect the fat layers, warm, filter through gauze & cool → "Enfleurage product" or "Floral pomade"

Enfleurage Process

Flower Petals

↓ Add fat mixture [Lard & tallow (2 : 1)]

1) Enfleurage Product (floral pomade)

[Fat saturated with oil]

↓
* Add absolute alcohol
* Triple extraction
* Cooling (remove most of fat)

2) Triple extract

[alc. solution of vol. oil + pigments + traces of fats]

↓

Evaporation of alcohol or fractional distillation	Dilution with H_2O + NaCl
↓	↓
3)Absolute of Enfleurage	**4)Volatile oil**
[Semi-solid, alcohol-free product]	

Jasmine flowers

“Enfleurage” Process

Cold Enfleurage

Hot Enfleurage

Super critical fluid extraction

Principle: based on using liquefied gases e.g. CO_2 under specific temperatures & pressures as extracting solvents. Under these conditions these gases are liquids but maintain the penetrating properties of gases & allow more efficient extraction. The oils obtained are of closest composition to the natural oils.

Process	Applications	Advantages	Disadvantages
Distillation	For dried & fresh material, rich in volatile oils with thermostable constituents	Cheapest method (apparatus, solvent & source of heat)	High temperature & presence of water may affect the constituents.
Scarification & Expression	For preparation of oils present in large amounts in outer peels of fruits & rich in heat-sensitive constituents.	-Carried at room temperature -Yields oils with more natural odors.	Expensive due to need of high number of workers
Extraction	Suitable for fresh material with heat-sensitive oils present in small amounts	-Carried at room or low temperature -Yields oils with more natural odors	Expensive due to use of solvent & / or high number of workers.