ESSENTIAL OILS '88

▶leaves	▶flowers	▶fruits	▶bark/wood	▶root/rhizome
Basil	Rose	Lime	Cinnamon	Vetiver
Mint	Jasmine	Beryamote	Sandalwood	Iris
Lemongrass	Ylang-Ylang	Orange	Amyris	Ginger
Geranium	Lavender	Lemon		
Patchouli				
Citronella				
Eucalyptus				
·ptus				

THAILAND INSTITUTE OF SCIENTIFIC AND TECHNOLOGICAL RESEARCH

REGIONAL WORKSHOP/SEMINAR ON ESSENTIAL OILS

ESSENTIAL OILS' 88

BANGKOK, THAILAND

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FOREWORD

The laboratory demonstration for the Regional Workshop/Seminar on Essential Oils (Essential Oils' 88) is designed to illustrate various techniques normally employed in the production, quality control and utilization of essential oils.

The essential oil production techniques will include steam, steam-water, water distillation and machine compression.

The quality control techniques will include some physicochemical methods as described in the British Standard 2073:1962 (UDC 668.5.017: 543) "Methods of Testing Essential Oils", the use of gas chromatograph and thin layer chromatograph.

Soft drink, food preparation and external use cream, incorporated with essential oils will be illustrated for the utilization of essential oils.

We hope that the laboratory demonstration, held within a limited time, will serve its purpose in promoting relevant knowledge and experience to participants which will prove useful in carrying out their duties.

CITRONELLA OIL

Introduction

Citronella oil is one of the most widely used natural essential oils. The oil is used extensively as a source of important perfumery chemicals like citronellal, citronellol and geraniol, which find extensive use in soap, perfumery, cosmetic and flavouring industries throughout the world.

There are two types of citronella: the more important type being the Java type produced from the grass Cymbopogon winterianus Jowitt. The less important one although longer established, is the Ceylon type distilled from the grass Cymbopogon nardus Bendle. The production of the latter takes place exclusively in Sri Lanka, the Java-type oil is produced elsewhere.

Citronella oil has a fresh lemon - like odour and is the source of geraniol and citronellal. Its principal application is in perfumery; its utilization in flavouring being insignificant.

The Ceylon-type oil accounts for only a very small proportion of world production and exports. The Java type, which accounts for the bulk of world production, is obtained mainly from Indonesia, China, Taiwan, India and Guatemala. The volume of world production is unknown but an international trade currently reaches 1,600-1,750 tons a year, of which Indonesia and China each contribute between 40% and 45%, Taiwan and Guatemala each a little under 3%, Sri Lanka about 7%, and Brazil 2%. The supplies are generally more than adequate for current demand.

Planting and cultivation

The plant is similar to lemongrass but leaf blades are broad, the stout is 2 m high. It is perennial herb with fibrous root. It flourishes best under tropical and subtropical climate. It needs abundant moisture and sunshine for good growth. Rainfall between 200-250 cm, well spread over the year, is ideal for the crop. It grows well in the range of altitude between 600-1,500 m.

The sandy loam soil with abundant organic matter is the most suitable. Heavy clay soils and sandy soils do not support good growth of the plant. The optimum pH of the soil is 6.

The citronella plants are propagated by splitting the chumps into slips. Each slip contains 1-3 tillers and is the unit of propagation. The slips should be set out 90x90 cm apart.

The suitable period of planting is during rainy season. The nitrogen fertilizer should be given at least 4 times per year, first month after planting and then after each harvest at the interval about three months. The recommended annual doses of nitrogen, P_2O_5 and K_2O are 80-120, 40, 40, kg/ha.

The citronella plantation should be kept weed-free, especially the newly established plantations and after each harvest.

Harvesting

The plant can be harvested after planting for 6 months. Only the leaves which contain maximum amount of the oil are harvested. The second and subsequent harvests can be taken thereafter $2\frac{1}{2}$ - 3 months interval. Harvesting too soon and too late affects the quality of oil adversely. The plantation can be harvested 4 times per year under the favourable conditions.

The same schedule of harvesting should be followed during second and subsequent years. Java citronella plantations remain productive for 5-6 years but the yield of leaves and oil is maximum during the second and third years, after which it starts decreasing. It is recommended that the plantation should be uprooted after 3-4 years and rotated with some small legume crops.

Distillation

Leaves are dried after harvesting because more dry leaves can be charged into a still and also, they require less steam and fuel. Time required for drying the grass depends upon the weather conditions about

3-4 hours in bright sunshine and a longer period during the rainy season. During drying, the leaves are turned over frequently with a view to prevent fermentation. The weeds are eliminated during the drying process. It is advisable that the dried material is distilled the same day it is harvested.

Ceylon type

A typical distillation post consists of two stills connected by one and the same condenser, which is inserted in a rectangular water basin made of concrete and imbedded in the ground. The stills, of galvanized iron, are usually 2.4 m high and 1.35 m wide; only a few are larger. The two stills are operated alternately--while one still is being charged or discharged, the other remains in operation. For this reason the two stills have only one helmet (with an attached gooseneck). Distillation is carried out with live steam generated in a separate and simple steam boiler, the latter partly imbedded in the ground. Warm water drawn from the condenser tank serves to feed the steam boiler. For heating, exhausted and sun-dried plant material is used. The ashes are dumped into the fields for fertilization purposes.

The oil separator and the container holding the separated oil are housed in a locked subterranean room, accessible through an underground passage, to which only the proprietor or trusted foreman has the key.

Distillation of one charge lasts about 6 hr. This rather protracted distillation is necessary because of the low steam pressure in the boiler, the limited capacity of the latter, and the comparatively large charge of plant material in the still.

Ceylon citronella contains approximately 0.5 per cent of oil. The yearly yield of dried grass per acre amounts to 4.5 tons of grass. The average yearly yield of Ceylon citronella oil per acre amounts to about 23 kg. The maximum yield of a plantation is obtained in the third year, at which time it reaches 27 or 36 kg of oil per year per acre. After the fifth year the yield diminishes.

Java type

Three types of stills are employed in Java--water and steam stills, steam stills, and the so-called "godokkans", a sort of intermediate between the first two types.

Water and steam stills. One of the advantages which water and steam distillation possesses over direct steam distillation is that the danger of steam channeling in the retort is diminished. The steam develops along the entire water surface, and consequently penetrates the charge of grass quite evenly.

The water and steam stills are imbedded into stone hearths and are provided with a grid, which supports the grass. The water beneath the grid is heated with direct fire. One manhole on top and one on the side permit charging and discharging of the plant material. The leaves are tramped into the still quite firmly, to prevent formation of steam channels; the volume of the charge diminishes greatly during distillation. Between the still walls and the plant charge especially in stills not properly insulated cooling and some internal condensation may take place and the condensate, which contains dark, non volatile extraction matter, collects beneath the grid, at the bottom of the still, and accumulates after a number of operations. It is advisable, therefore, to draw off the water in the still (beneath the grid) after each charge has been distilled, and to replace it with fresh water for the distillation of the next batch.

The ratio of the diameter of a retort to its height should be at least 1 to 1.5 or 2. Steam channels are more readily in low, wide retorts than in narrow and high ones, but danger is remedied if a wide retort tapers conically toward the top. Such stills, although being highly superior, are not yet being used very much in Java. It is highly advisable to insulate the retort with a layer of kieselguhr or other material; even finely chopped grass can be used. A retort of 1.10 m diameter and 2.40 m height holds 485 to 546 kg of grass, which is quite sufficient for one charge.

Water and steam distillation requires a separate fire for each still. Thus, the fuel consumption is relatively greater than in one separate steam boiler, which supplies steam for the whole battery system. Water and steam distillation (with direct fire) can thus be recommended only if small quantities of grass are processed daily, and if the price of fuel is low, as in the case of exhausted grass. Operating a retort holding 485 kg of grass, by water and steam distillation, requires 3 to

4 hr, which is twice as long as would be required with direct steam distillation.

Distillation by the godokkans process. Distillation by using the so-called godokkans represents a modified version of water and steam distillation. This type of equipment is usually owned by wealthier natives or Chinese. The fire hearth and the water boiler are separated from the retort. The capacity of the godokkans varies from 400 to 4,000 kg of grass. A retort holding more than 1,500 kg of leaves is less economical than that holding 1,000 to 1,200 kg. Retorts of small capacity require a shorter distillation time. It has been the practice to distil a charge of 1,000 to 1,200 kg for 5 to 6 hr, although 3 to 4 hr is preferred.

Distilling with direct steam. In the case of direct steam distillation, the steam is generated in a separate boiler heated by wood or exhausted grass. The retort resembles that of water and steam stills. As regards the question of steam pressure, it was formerly believed that a high steam pressure would give best results. Recommending a pressure of 3 to 4 atmospheres (measured in the steam boiler), but later the producers came to realize that such high pressure, while giving a greater yield, affected the quality of the oil adversely. Instead of 3 to 4 atmospheres steam pressure, 1 to 2 atmospheres are then recommended. The loss in yield of oil obtained by the use of low pressure steam is more than compensated by a high total geraniol content of the oil. If the distillation is continued sufficiently long all the volatile oil present in the plant will be distilled. Such oil, however, contains also the less desirable constituents. At present, a direct steam still holding 1,000 to 1,200 kg of leaves is operated with steam of $\frac{1}{2}$ to 1 atmosphere (above atmospheric pressure, measured in the steam boiler) throughout the distillation, which lasts not longer than 3 hr. The length of distillation obviously depends not only upon the steam pressure, but also upon other factors such as condition (moisture content) of the plant material.

It is difficult to express the yield of oil per acre of grass in definite figures because it depends upon so many factors, viz. climate, fertility of the soil, age of the planting, and method of distillation. The figure on yield estimated when the first citronella estate was established in Java on normal soil during the second year corresponded to about 14 tons per acre. The oil yield average of 0.7 per cent, works out at 97 kg per acre.

Physico-chemical properties

The odour of the Ceylon citronella oil is coarser than that of the Java oil. Its content of total geraniol and citronellal is substantially lower than that of the Java oil, which is responsible for the lower quality and value of the Ceylon oil.

British standard specification (BS 2999/18-19:1972) defines certain characteristics of oil of Ceylon and Java citronella in relation to the assessment of its quality as follows:

	Ceylon Citronella oil	Java Citronella oil
Apparent density 20°C	0.893-0.910 g/ml	0.880-0.892 g/ml
Optical rotation 20°C	-9° to -18°	0° to -5°
Refractive index 20°C	1.479-1.485	1.466-1.473
Solubility in ethanol 80% (v/v)	1:4	1:2
Carbonyl value*	25-55	127
Ester value after acetylation+	185-201	251

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*127 - corresponding to 35% of carbonyl compounds, expressed as citronellal.

+250 - corresponding to 85% of constituents liable to acetylation, expressed as geraniol.

Chemical composition

The main differences in chemical composition both qualitative and quantitative between Java and Ceylon citronella oil were the following:

Compound	Ceylon Citronella oil	Java Citronella oil
Tricyclene	1.6	-
α - Pinene	2.6	-
Camphene	8.0	-
β - Pinene	trace	-
Sabinene	trace	-
Myrcene	0.3	-
Car-3-ene	trace	-
α - Phellandrene	0.8	-
Limonene	9.7	1.3
cis-Ocimene; α - Terpinene	1.4	-
trans - Ocimene; β - Phellandrene	1.8	-
p - Cymene	trace	-
Terpinolene	0.7	-
1 - Hexanol	0.1	-
Methyl heptenone	0.2	trace
Citronellal	5.2	32.7
Camphor	0.5	-
Bourbonene	1.0	trace
Linalool	1.2	1.5
Linalyl acetate	0.8	2.0
α - Terpineol	trace	-
β - Caryophyllene	3.2	2.1
4 - Terpineol	0.7	trace
Menthol	trace	-
Citronellyl acetate	1.9	3.0

Compound	Ceylon Citronella oil	Java Citronella oil
β - Caryophyllene	3.2	2.1
4 - Terpineol	0.7	trace
Menthol	trace	-
Citronellyl acetate	1.9	3.0
1 - Borneol	6.6	trace
Geranyl formate	4.2	2.5
Citronellol; Geranyl acetate	8.4	15.9
Nerol	0.9	7.7
Geraniol	18.0	23.9
Citronellyl acetate	trace	trace
Geranyl butyrate	1.5	-
Nerolidol	0.3	-
Methyl eugenol	1.7	trace
Elemol	1.7	6.0
Methyl iso-eugenol; Eugenol	7.2	2.3
Unidientified	1.5	1.4
Farnesol	trace	0.6

Use

Ceylon citronella oil is used chiefly for the scenting of soaps, sprays, disinfectants, polishes, and all kinds of technical preparations where price is of prime consideration.

Citronella oils, Java type, is one of the most important essential oils. Oil with a high content of citronellal is used chiefly for the isolation of citronellal, and for conversion into citronellol, citronellol esters, hydroxy citronellal, and synthetic mentol. Oil with a low citronellal and high geraniol content is used for the extraction of geraniol, and conversion of the latter into esters. The price of this type of oil is lower, and thus serves well for the scenting of soaps and technical preparations where cost is of prime consideration. In many cases it can replace the Ceylon type of citronella oil. The bulk of Java type citronella oil, however, is of the usual, normal quality, i.e., comprising 35 per cent of citronellal and 85 per cent of total geraniol. The oil is used for the extraction of aromatic isolates, as well as for the scenting of soaps and all kinds of technical preparations.

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LEMONGRASS

Introduction

The two main types of lemongrass are recognized by the trade. The "East Indian" oil is obtained from *Cymbopogon flexuosus* (DC.) Stapf., cultivated in Travancore near the Malabar coast; the "West Indian" lemongrass oil is obtained from *Cymbopogon citratus* (DC.) Stapf., cultivated in the tropics, particularly in Guatemala, where production is organized on a considerable scale and mechanization is used. Lemongrass oil is one of the most important essential oils. The world - production is 800-1,300 tons per year, produced from India, Guatemala, China. The price is around £ 9 per kg for cochin 75%.

The oil has typical strong lemon like odour of the plant due to high citral content (75-90%). Among the two species cultivated, *C. flexuosus*, also known as Malabar or Cochin Grass, is indigenous to India, whereas *C. citratus* is cultivated in West Indies, Guatemala, Brazil, etc. The oil of *C. citratus* is considered inferior as it contains slightly less citral (60-75%), whereas oil from *C. flexuosus* contains more citral (75-90%).

In Thailand a variety of *C. citratus*, locally called takhrai, is widely grown in the garden. It is also used in flavouring certain soups and curries.

Planting, harvesting and distillation

Lemongrass requires a warm and humid climate with plenty of sunshine and rainfall, ranging from 250 cm to 300 cm, uniformly distributed over the year. It is hardy plant and resistant to drought.

It grows well at altitudes between 100 and 1,200 m, generally on poor soils along with hill slopes, but flourishes on a wide variety of soils ranging from rich loam to poor laterite. The grass grows best on well-drained sandy-loam soil; it even thrives on light-sandy soils, provided they are well manured. Plants from such a sandy soil yield relatively more oil with higher citral content than plants from very fertile soil.

The plants are propagated both from seeds and rooted slips in case of C. flexuosus, whereas in case of C. citratus, it is propagated through rooted slips. Lemongrass is a soil-exhausting crop. It is preferable to use spent lemongrass in the form of compost at the rate of about 10 tons per ha and wood ash at the rate of about 2 tons per ha. Weeding and hoeing are very important as they affect the yield and quality of oil.

The time of harvesting affects the yield and quality of the oil. Young and tender grass, harvested in the early season, gives an oil which is low in aldehyde content (60-70%) and of poor solubility. Later, the aldehyde content of the oil increases to 75% and more. The yield of oil also increases. The first harvest is generally possible after 3 months of transplanting in case of C. flexuosus and after 6 months of planting of C. citratus. Subsequent harvests take place at intervals of 40-50 days depending upon the fertility of the soil and seasonal factors. Under normal conditions, three harvests are possible during the first year and four in subsequent years. Harvesting is done with the help of sickles, the plant being cut close to their bases about 10 cm above ground bud.

The grass is either distilled fresh or allowed to wilt for 24 hours. Wilting reduces the moisture content and allows larger quantity of grass to be packed into the still, thus economising the fuel use.

For good quality oil, it is advisable to use steam-distillation. To obtain the maximum yield of oil and facilitate release of oil, the grass is chopped into shorter lengths. Chopping the grass has further advantages that more grass can be charged into the still and even packing is facilitated. The grass should be packed firmly as this prevents the formation of steam channels. The steam is allowed to pass into the still with a steam pressure from 18 to 32 kg in the boiler.

The yield of grass is the lowest in the first year and highest in the third year and fourth year after planting. On an average, one hectare produces 75-100 kg of oil. The percentage yield based on fresh weight varies between 0.2 and 0.4.

Physico-chemical property

Oil of lemongrass is a mobile, yellow to reddish-brown oil with a powerful lemon-like odor.

The physico-chemical property is as followed:

	East Indian	West Indian
	B.S. 2 999/32-43	B.S. 2 999/32-43
	1 963 : 2 999/35	1 963 : 2 999/36
Specific gravity at 20°C	0.893-0.903	0.870-0.895
Optical rotation	- 3 - (+ 1)	- 3 - (+ 1)
Refractive index at 25°C	1.483-1.489	1.483-1.489
Solubility in 70% v/v alcohol at 26°C	Not less than 75	Not less than 75
Carbonyl value	Not less than 276	

Chemical composition

The main constituent of lemongrass oil is citral ∼ 75-85%. Other minor constituents present in oil are :

Linalool
Geraniol
Geranyl acetate
Farnesol
Farnesal
Citronellal
Citronellol
Limonene
Myrcene
Nerol

Use

Because of its powerful lemon-like odour, oil of lemongrass is used widely for the scenting of soaps, detergents and all kinds of technical products. The bulk of the oil, however, is employed for the isolation of citral, which, as such, is used in flavors, cosmetics and perfumes, or is converted into ionones, a group of very important synthetic aromatics possessing a strong and lasting violet odour.

As the starting material for the preparation of the ionones, oil of lemongrass has become one of the most important and undispensable essential oils in the course of the past fifty years. This applies to the East Indian as well as to the West Indian type of lemongrass oil. Large quantities of β -ionone are now employed for the manufacture of synthetic vitamin A.

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ROSE OIL

Introduction

Rose oil is one of the oldest and best known scents used in perfumery. Rose oil is obtained from various species of Rosa. Rosa is a large genus of erect, sarmentose or climbing shrubs, widely distributed in the temperate regions of the northern hemisphere and on tropical mountains. Three, out of the various species of Rosa, have been used commercially for production of essential oil.

1. Rosa damascena is the most high grade oil and the major producers of this oil are Bulgaria, Turkey and India.

2. Rosa gallica is the limited extent of oil, produced in USSR and Egypt.

3. Rosa centifolia is the third species of Rosa which the oil is produced in France and Morocco.

Here, we would only mention, R. damascena, the species which is native to the Asiatic region. R. damascena is a perennial with a life span up to fifty years, 2.5-3.0 m high. The stems are somewhat arching with numerous stout and hooked falcate prickles of unequal size, intermixed with numerous unequal aciculi and glandular bristles. Leaf stipulate, compound, imparipinnate with 5-7 leaflets; stipules adnate; leaflets moderately large 2.5-7.5 cm long and 1.5-4.0 cm broad, ovate to oblong, glabrous on the upper surface but softly pubescent at the lower end, margin serrate, glandular with fine hairy outgrowths which are also present on the raised midrib of the lower surface; glands brown to red; petiolules also pubescent and slightly glandular. Inflorescence, corymbose; flowers sweet-scented, red, pink or white sometimes striped; pedicel possesses densely packed aciculi and hispid glands; bracts more or less lanceolate; sepals reflexed; petals variable in color, white or red. Gymoecium apocarpous. Fruit ovoid or obovate.

Planting and cultivation

Rose is propagated vegetatively only as the plants obtained from seed usually have a smaller number of petals.

The ground must be well ploughed before planting, parallel ditches, 3.5 ft deep and 1.5 to 1.75 ft wide, and 7 to 8 ft apart, are dug. The earth shovelled up from the ditches is piled in equal amounts on both sides of the ditch. The work should be undertaken for not less than 2 months prior to planting, so that the earth can be exposed to air and moisture sufficiently long for the organic matter to decompose. At the time of planting, a part of the earth piled up along the ditch is shovelled back so that the ditch has now a depth of only 1.5 to 2 ft (the deeper the bushes are planted, the better they will withstand periods of drought). The cutting necessary for planting are procured from an old rose field. Cuttings 1 to 2 ft long are taken from the base of healthy old rose bushes, the upper, green parts being removed. The woody cuttings are placed into the ditches horizontally, in four uninterrupted rows, 2.5 to 3 in apart. Then a layer of earth (from the piles along the ditches), 2 to 3 in thick, is placed on top of the cuttings; this is followed by 2 in of seasoned stable manure. Two to three months later, the first shoots appear above ground. The soil is then slightly hoed, weeds are removed, and some more earth is shovelled into the ditches and placed around the young plants. During the first year this procedure (hoeing, weeding, and placing of earth around the young plants) has to be repeated at least eight times. In the second and third year the field must be hoed and ploughed five times each year. After ten years the plantation may show signs of decay; the bushes are then cut down to the ground for rejuvenation. If properly taken care of every ten years a field may remain productive from thirty to forty years. However, such care requires a great deal of work. Weeds must be removed, old branches cut out, and plenty of manure applied,

Harvesting

Blooming date and duration of the crop depend a great deal upon the weather prevailing in a particular year. Mild and humid weather prolongs the flowering period, increasing at the same time the yield of oil and producing an oil of good quality. But during very hot and dry weather the harvest may last only two weeks, and the yield of oil may be lowered due to loss by evaporation. Moreover, the quality of the oil is also affected because of the large quantities arrival of roses at the distilleries cannot be worked up immediately and may be left lying on the floor for hours. During this interval they are likely to start fermenting or at least to turn so stale that their aroma suffers gravely.

Generally, harvesting begins as soon as the flowers begin to open and continues until all have been picked. Flowers are collected by hand, being nipped off just below the calyx. Gathering of the flowers starts at daybreak and ceases at eight or nine in the morning. When collected early in the morning, the roses give a much higher yield of oil than when gathered in the afternoon. The harvesters drop the flowers into bags and then transfer ~~them~~ into sacks, which are hauled to the distilleries as soon as possible.

Distillation of rose oil

Distillation is carried out in steam stills. The flowers are covered with water and they should move freely in it. Start distillation carefully and slowly. Time of distillation depends upon the capacity of the still. The inlet-cooling water temperature in the condenser should be between 35°-45°C. Since at lower temperature, a stereoptene present in the oil solidifies in the condenser tubes. The volumes of water distilling over should be just sufficient to carry out the oil and not excess. Too much aqueous distillation washed out the oil separating in the separator and redissolves some of its constituents. Only 20% of the oil is obtained in first distillation and all the distillate is passed through a cohobation chamber in order to get the rest of oil.

Yield and oil content

The yield and quality of oil depend upon various factors, such as soil and climate, season of harvest, time of plucking the flowers during the day, condition of flowers used, e.g. fresh or pretreated type of still used etc.

Yields varying from 0.02 to 0.03%.

Physico-chemical properties

Specific gravity at 30°	0.8485 to 0.8605
Optical rotation	$-2^{\circ}\,18'$ to $-4^{\circ}\,2.4'$
Refractive index at 25°	1.4530 to 1.4640
Freezing point	$+16.5^{\circ}$ to $+22.5^{\circ}$
Acid value	0.92 to 3.75
Ester value	7.2 to 17.2

Chemical composition

Major constituents of Bulgarian rose oil are rhodinol (1-citronellol) and geraniol. The following table gives the relative proportion of major constituents of Bulgarian rose oil:

Rhodinol (1-citronellol)	38%
Paraffins	16%
Geraniol	14%
Nerol	7%
β-Phenyl ethanol	3%
Eugenol methyl ether	3%
Linalool	2%
Farnesol	1%

Uses

Rose oil imparts characteristic flowery top notes to perfumes. The extracted absolute adds lasting tonalities and increases fixation of the aroma. So a mixture of distilled oil and extracted absolute shows the advantages of these characteristics. The distilled oil is used where solubility in dilute alcohol is important. The absolute is limited only in high proof alcohol, therefore it can be used as perfume in the products where solubility plays no role. In powders, cream and even the lower priced products, rose concrete gives excellent results.

References:-

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CITRUS HYSTRIX

MACHINE-PRESSED OIL OF CITRUS HYSTRIX DC. PEEL

Introduction

Citrus hystrix DC. or "makrut" as it is known in Thailand, is a source of essential oils which could be some importance to the food and cosmetic industries. The oil is used as a flavour in shampoo.

The essential oil obtained from C. hystrix peel by cold-pressed method.

The quality of the cold-pressed citrus oil is considered superior to the oil extracted by other methods. Cold compression does not alter the aromatic constituents of the oil and thereby keeps the fragrance and flavour value intact. Pressed oil may be used in perfumes and flavours for its refreshing, sweet-fruity note. The fruit juice is used in food, Thai traditional drug and shampoo preparation. The essential oil is used as stimulant and stomachic.

Chemical constituents of Citrus hystrix DC. peel oil are 2-pinene, camphene, β -pinene, sabinene, myrcene, limonene, trans-ocimine, α -terpinene, ρ -cymene, citronellal, linalool, etc. The high terpene content in pressed oil accounts for less solubility in aqueous-alcohol solution, and becomes a problem in making flavour or perfume compounds. Vacuum distillation can remove those readily oxidizable constituents, and thus improve the stability and solubility of the oil.

Physico-chemical property

	Cold-pressed oil
Specific gravity at 20°C	0.8944
Refractive index	1.4825
Ester value	48.23
Acid value	4.74

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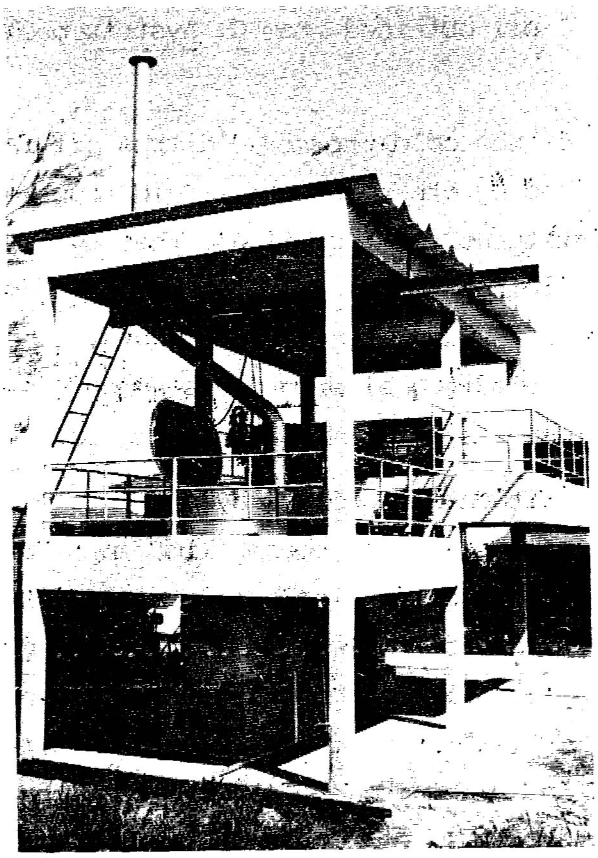

Figure 1. Water-steam still

APPENDIX I

Essential Oil Production

Commercial Scale

Type Water-steam distillation

Capacity 3,000 l or wilt materials 600 kg

Source of energy Diesel oil

Raw material Citronella (*Cymbopogon winterianus*)

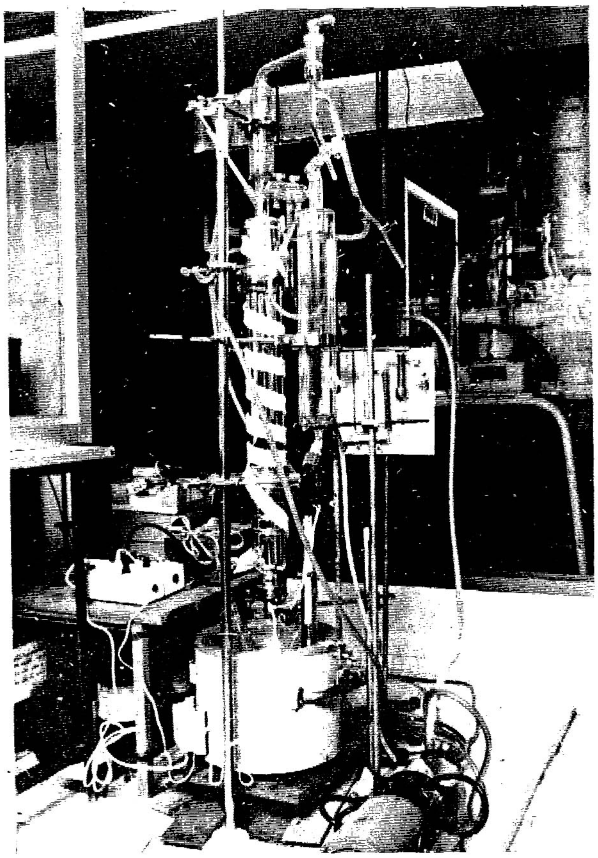

Figure 2. Fractional distillation apparatus.

APPENDIX II

FRACTIONAL DISTILLATION, CITRONELLA, CYMBOPOGON WINTERIANUS

Purpose To isolate various fractions of Java citronella oil.

Introduction The separation of volatile oil into various fractions is obtained by method of fractional distillation. The process must be carried out under reduced pressure since the oil tends to decompose and resinify at high temperature. Furthermore, an effectively fractionating column, absolutely airtight joints, and also an efficient vacuum still, are required in order to collect the desired fractions of volatile oil.

Experiment Place 500 ml of citronella oil in a 3-necked round bottom flask fitted with an air-leak tube, a thermometer and a fractionating column. The column, packed with stainless steel wire-mesh rings, is connected to a column head. Adjust the reflux by means of a column head while reducing pressure. Collect oil at the desired pressure and temperature. Determine the composition of the fractions by GLC.

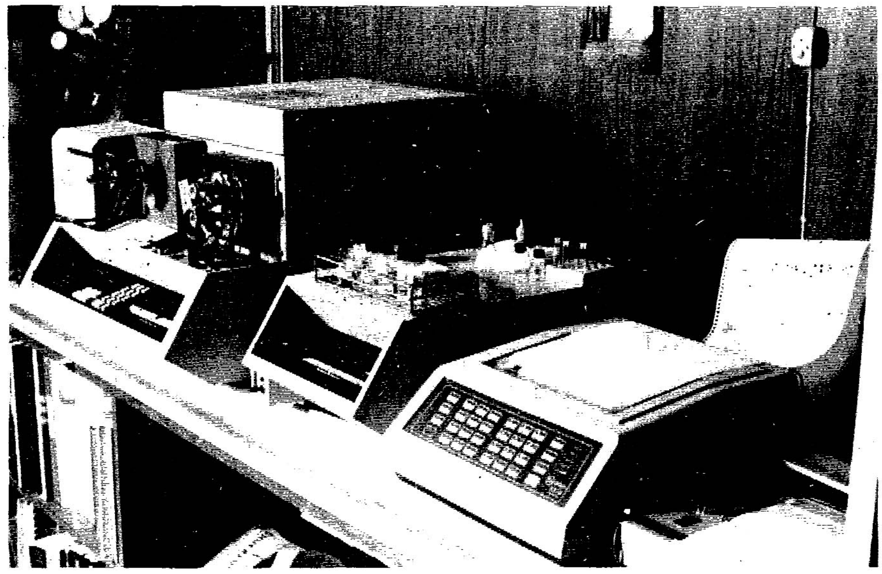

Figure 3. Gas chromatograph.

APPENDIX III

GAS CHROMATOGRAPH

The citronella oil is very often adulterated with some miscible liquids. Some physico-chemical methods could not show the difference of the adulterated oil to the genuine oil. Gas chromatography, however, could be employed reliable to detect and determine the adulterants in the oil. In this experiment, both adulterated and genuine oil are injected simultaneously into the injector parts of the gas chromatograph. The vaporized oil will pass through two columns to the detectors. The signal from each detector is then separately amplified to drive a two-pen recorder system. Compare the two chromatograms and note the operating condition.

Sample

Description
Size

Column

Details
Temperature

Detector	Instrument
Type	Varian Aerograph Series
Temperature	Recorder
Attenuation	Varian Aerograph Model 1 mV recorder, speed
Injector Temperature	Operator
Relative N_2 flow rate	Date

Note

APPENDIX IV

THIN LAYER CHROMATOGRAPHY OF ESSENTIAL OILS

The adulteration of volatile oil by the addition of cheaper grades of the same oil or of the cheaper oil having a similar odour is sometimes practiced. The detection of adulteration in volatile oil is difficult to make which may not be detected by commonly used chemical and physico-chemical methods. The sense of smell and taste applied to the dilution of the oil in various media can be used but this **organoleptic** test needs the very skilful person. The gas chromatography method has been used for the study of finger prints and comparing with that of the standard oil(s).

In this experiment, small quantity (less than 3.0%) of citronella oil is adulterated in geranium oil. It is very difficult to detect the adulteration by using the gas chromatography method since the major component, citronellal peak, of citronella oil has the same retention time of the unidentified peak in geranium oil. Thin layer chromatography technique is applied in this experiment which can show the differences of geranium and citronella oil. At least three spots are different in colour.

The small quantity between 1.5-3.5% of citronella oil is adulterated in geranium oil and diluted with hexane to contain 25% oil concentration. The quantity applied on the silicagel GF_{254} plate is 0.5-1 µl. The solvent system, hexane/ethyl acetate (90/10) is used and run for 30-35 minutes. The sprayed reagent is 1.0% vanillin in ethyl alcohol which contain 2.0% of concentrated sulfuric acid. Record the spots appear in the cold and after place in an oven at 100-105°C for 7-8 minutes.

The authentics available are linalool, citronellol, geraniol and citronellal.

Reference

PARIS, R. and GODOM, M. : "Thin-layer paper chromatography of essential oils". Recherches, No. 13, 1963, pp. 48-77, Societe Anonyme Des Etablissements, Roure Bertrand Fils Et Justin Dupont, Paris, France.

APPENDIX V

Semi-pilot Scale

Type Steam distillation

1. Capacity 450 l or wilt materials 75 kg

   Source of energy Steam

   Experiment Steam

   Raw material lemongrass (*Cymbopogon citratus*)

2. Capacity 160 l or wilt materials 25 kg

   Source of energy Steam

   Experiment Steam

   Raw material

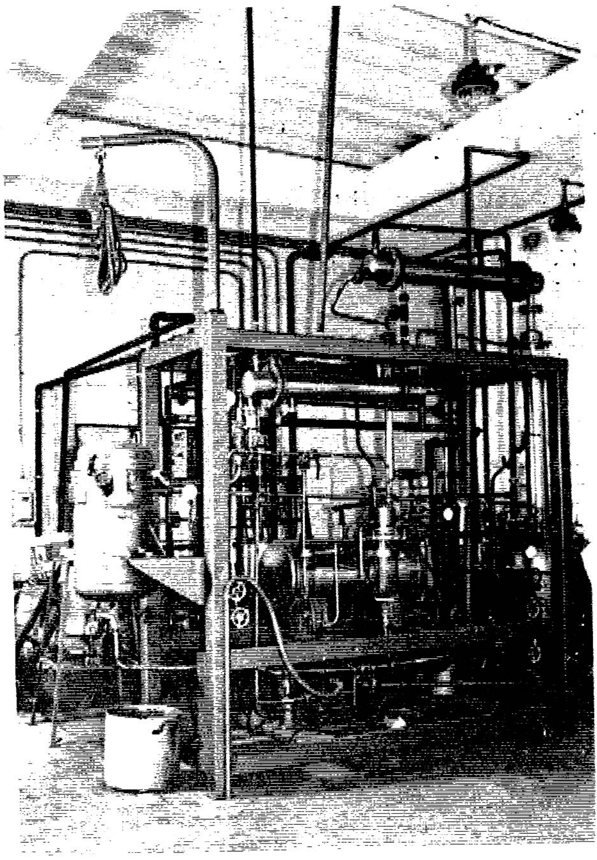

Figure 4. Versatile extractor unit.

APPENDIX VI

Laboratory Scale Versatile Extractor

Type Water distillation, Versatile Extractor

Capacity 80 l

Source of energy Steam

Raw material *Rosa damascena*, fresh flowers 4 kg

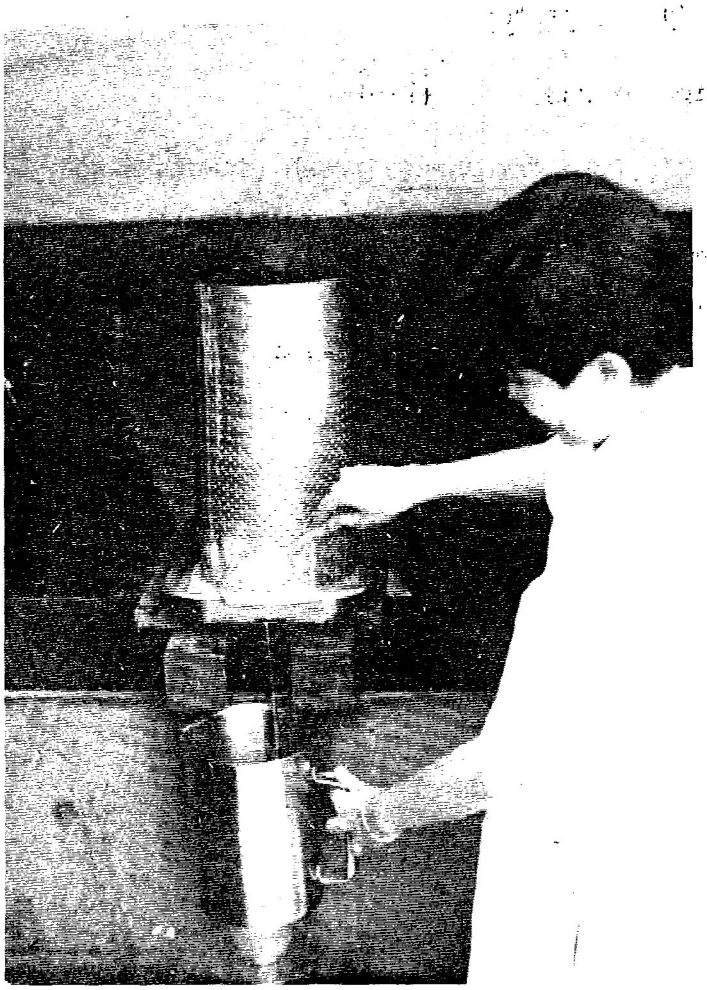

Figure 5. Machine-pressed oil of *Citrus hystrix* peel.

APPENDIX VII

MACHINE-PRESSED OIL OF CITRUS HYSTRIX PEEL

Method Hydraulic machine is employed for oil compression. The green fruits are thoroughly washed, the peel is removed from the fruit by knife and a small amount of water is added before compression. The excluded essential oil is washed away by water spraying; the oil separated from the water and other residuals by centrifugation.

By water distillation of the machine-pressed oil, the colorless essential oil is obtained.

Gas chromatography

Each fraction obtained is analysed by gas chromatograph. Some chemical constituents in the fraction are also determined by comparing with the standard components under the same condition. The operating conditions are noted below.

Sample	distilled Citrus hystrix oil
Column	5% SE 30 on CHROMOSORB M 6 ft long
Temperature	70-200°C
Injector temperature	230°C
Detector temperature	230°C

The chemical composition of the oil is as follows:

alpha-pinene
camphene
beta-pinene
sabinene
myrcene
limonene
1, 8 cineole
gamma-terpinene
cymene
terpinolene
trans-sabinene hydrate

citronellal
linalool
caryophyllene
alpha-terpineol
geraniol
geraniol acetate
citronellol

Reference:

1) ARCTANDER, Steffen (1960) "Perfume and Flavour Materials of Natural Origin" (Published by author : New Jersey.)

2) LAWRENCE, B.M., HOGG, J.W., TERHUNE, J.S. and PODIMUANG, V. (1970) "Report No. 1, The Leaf and Peel oils of Citrus Hystrix DC."

APPENDIX VIII

METHODS OF TESTING ESSENTIAL OILS

(B.S. 2073:1962)

PREPARATION OF THE OIL FOR EXAMINATION

Remove, as far as possible, any visible water present in the sample, add about 15 per cent by weight of freshly ignited, neutral magnesium sulphate and shake the mixture vigorously from time to time during a period of two hours; filter through paper.

In the case of oils which are solid or partly solid at room temperatures (e.g. oils of rose, guaiac wood or araucaria), warm the oil just sufficiently to liquefy it and proceed as above, maintaining the temperature such that the oil remains liquid throughout.

The colour of some dark-coloured oils may be reduced by shaking the oil intermittently for a period of 10 minutes with 1 per cent by weight of tartaric or citric acid. Filter and proceed as above.

PHYSICAL METHODS

DETERMINATION OF SPECIFIC GRAVITY (in air)

a. Definition. The specific gravity (S t/20°C in air)[*] of an essential oil is the ratio of the weight in air of a given volume of the oil at t°C[+] to that of the same volume of water at 20°C, the weighing being made with weights adjusted to balance brass weights in air.

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[*] The above definition of specific gravity is commonly used for essential oils and differs from those used in other fields, for example that in B.S.718,[‡] and in the petroleum industry. The term adopted by the International Organization for Standardization is 'relative density with reference to water'. The ISO definition relates to the ratio of the true densities and not to the ratio of the weights in air.

[+] t°C = 30°C for oils of rose, araucaria and guaiac wood; 20°C for other essential oils.

[‡] B.S. 718, 'Density hydrometers and specific gravity hydrometers'.

b. Procedure. Calibrate a specific gravity bottle[*] or pycnometer (of capacity at least 10 ml) as follows:

Clean and dry the bottle or pycnometer and weigh it; fill it with freshly boiled and cooled distilled water and keep it in a bath of water at 20°C until it reaches that temperature. If a bottle is used, insert the stopper in such a way that the capillary is completely filled with water, and then maintain it at 20°C until no further alteration in volume occurs. Wipe the stopper. If a pycnometer is used, adjust the volume of liquid to the fixed mark. Remove the bottle or pycnometer, dry the outside, and weigh.

Empty and dry the bottle or pycnometer. Fill it with the sample of oil previously brought near to the temperature t°C. Keep the bottle or pycnometer in a bath adjusted to t°C until it has attained that temperature. If a bottle is used, insert the stopper in such a way that the capillary is completely filled with the oil and then maintain it at the temperature, t°C, until no further alteration in volume occurs. Wipe the stopper. If a pycnometer is used, adjust the volume to the fixed mark. Remove the bottle or pycnometer, dry the outside, and weigh.

c. Calculation. Specific gravity (S t/20°C) in air $= \dfrac{W_2}{W_1 [1+\alpha(t-20)]}$

where W_1 = weight, in grammes, of water obtained in calibration test,
where W_2 = weight, in grammes, of oil obtained in the test,
and = coefficient of cubic expansion of glass at the given temperature;
= 0.000 03 for soda glass;
= 0.000 01 for borosilicate glass.

NOTE. Specific gravity at a given temperature, S t°/20°C, may be converted to apparent density (see page35) at this temperature, t°C, by multiplying by the factor 0.997 18. The appropriate correction for a series of specific gravities is given in Table 1.

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[*] Suitable bottles are described in B.S. 733, 'Density bottles'.

TABLE 1. CORRECTIONS FOR CONVERTING SPECIFIC GRAVITY TO APPARENT DENSITY

Specific gravity S t/20°C	Correction to be applied to give apparent density (g/ml) at t°C
0.75	- 0.002 1
0.80	- 0.002 3
0.85	- 0.002 4
0.90	- 0.002 5
0.95	- 0.002 7
1.00	- 0.002 8
1.05	- 0.003 0
1.10	- 0.003 1
1.15	- 0.003 2
1.20	- 0.003 4

DETERMINATION OF APPARENT DENSITY
(WEIGHT PER MILLILITRE) (at t°C in air).

a. **Definition.** The apparent density (weight per millilitre) of an essential oil at t°C is the weight in air, in grammes, of one millilitre of the oil at t°C* against weights adjusted to balance brass weights in air.

b. **Procedure.** Determine the capacity of a density bottle+ or pycnometer (of capacity at least 10 ml) from the fact that, when weighed against brass weights in air of density 0.0012 g/ml, 1 litre of freshly distilled water at 20°C weighs 997.18 g or at 30°C weighs 994.62 g. Ordinary deviations in the density of air from the mean value of 0.0012 g/ml do not affect the result.

Fill the bottle or pycnometer with the oil and keep the temperature at t°C. Weigh the filled bottle or pycnometer in air.

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*t°C = 30°C for oils of rose, araucaria and guaiac wood; 20°C for other essential oils.

+A bottle complying with B.S. 733, 'Density bottles', is suitable.

c. Calculation. Apparent density $= \frac{W}{V}$ g/ml,

where W = weight, in grammes, of the oil at t°C,
and V = capacity, in millilitres, of the bottle or pycnometer at t°C.

NOTE. Apparent density at a given temperature, t°C, may be converted to specific gravity at this temperature t/20°C by multiplying by the factor 1.002 83. The appropriate corrections for a series of apparent densities are given in Table 2.

TABLE 2. CORRECTIONS FOR CONVERTING APPARENT DENSITY TO SPECIFIC GRAVITY

Apparent density (g/ml) at t°C	Correction to be applied to give sp.gr. (S t/20°C)
0.75	+ 0.002 1
0.80	+ 0.002 3
0.85	+ 0.002 4
0.90	+ 0.002 5
0.95	+ 0.002 7
1.00	+ 0.002 8
1.05	+ 0.003 0
1.10	+ 0.003 1
1.15	+ 0.003 3
1.20	+ 0.003 4

DETERMINATION OF OPTICAL ROTATION

a. Definition. For the purpose of this determination the optical rotation of an essential oil is taken as the angle in degrees through which the plane of polarization is turned when plane-polarized sodium light is passed through a layer of oil, 1 dm in thickness.

The notation is optical rotation α_D^t, t being the temperature (°C) at which the determination is made.

b. General. The determination should be carried out in a dark-room, using sodium light. Any recognized type of polarimeter may be used. The standard temperature for the expression of results is 20°C.

When the determination is carried out at any other temperature, this temperature should be recorded together with the observed rotation. This is particularly important in the case of oils of high optical rotation.

c. Procedure. Switch the light source on and wait until full luminosity is obtained. Fill the polarimeter tube with the essential oil at the required temperature, ensuring the absence of air bubbles. Place the tube in the polarimeter; read the dextrorotatory (+) or laevorotatory (-) optical rotation of the oil on the scale of the instrument. Allow ample time for the oil and observation tube to attain a steady temperature before taking readings.

As far as possible make determinations using 1 dm tubes. In the case of dark-coloured oils or oils having a high rotation, e.g. above 90°, a shorter tube may be used, whilst with light-coloured oils or oils low rotation the determination may be made in a longer tube, calculating the results to a length of 1 dm in each case, The tolerance in length on all tubes shall be ± 0.05 mm.

d. Expression of results. Record the results in degrees. Take the average of at least three readings, which should agree within 0.08°, and round to the first decimal place.

DETERMINATION OF REFRACTIVE INDEX

a. Definition. For the purpose of this determination, the refractive index of an essential oil is taken as the ratio of the sine of the angle of incidence to the sine of the angle of refraction when a ray of wave-length 589.3 nm* (the mean of the D lines of sodium) passes from air into the oil.

The notation is refractive index n_D^t, t being the temperature (°C) at which the determination is made.

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*1 nanometre (1 nm) = 10^{-9} metre = 1 millimicron (1 mµ).

b. Procedure. In order to obtain a preliminary indication, cool a few millilitres of the oil in a small test tube and stir with the thermometer until solidification takes place, note the temperature and set aside the tube of solidified oil in a cool place. Then fill the outer container of the apparatus with water (or brine) at a temperature about 5 degC lower than that noted above, and fit the larger outer tube in its place. Into the inner tube place 10 ml of the oil, insert the thermometer and cool the tube and oil to the temperature indicated in the preliminary test. Now insert the tube and contents in the apparatus, and allow the temperature to fall a further 1 or 2 degC. Then seed the oil with a trace of the previously solidified oil and stir with the thermometer until solidification takes place.

Record the highest temperature reached as the freezing point.

DETERMINATION OF SOLUBILITY IN ETHANOL

a. General. All essential oils are soluble in absolute ethanol, and many are soluble in diluted ethanol. Frequently, however, the solutions obtained are not clear, and are described as 'opalescent'.

b. Definitions. The terms used for describing the solubility of essential oils are as follows:

Soluble, or completely soluble, means that the oils form a clear and bright solution in the proportions stated.

Soluble with opalescence means that the solution formed is not entirely clear and bright, but its opalescence does not exceed that of the reference opalescence prepared as described below.

c. Reagents. Ethanol, diluted. The following table shows the strengths, as percentages by volume, of the aqueous ethanolic solutions usually employed in the determination of solubilities, together with the corresponding tolerances for relative density, measured at 20/20°C.

TABLE 3. RELATIVE DENSITY OF AQUEOUS ETHANOL SOLUTIONS

Ethanol (per cent v/v)	Relative density limits
95	0.812 3-0.813 0
90	0.830 3-0.830 9
85	0.846 0-0.846 6
80	0.860 4-0.860 9
75	0.874 0-0.874 5
70	0.886 8-0.887 3
65	0.899 0-0.899 4
60	0.910 5-0.910 9
55	0.921 4-0.921 9

Solution of reference opalescence, freshly prepared by adding 0.5 ml of 0.1N silver nitrate solution to 50 ml of 0.0002N sodium chloride solution and stirring. Add one drop of diluted (25 per cent) nitric acid and observe after 5 minutes. Shield from direct sunlight.

d. Procedure. Place 1 ml[*], accurately measured, of the oil in a 10 ml or 25 ml stoppered graduated cylinder, and add the ethanol of appropriate strength according to the oil being tested, drop by drop, shaking after each addition, until as clear a solution as possible is obtained at a temperature of 20°C.

If the solution is not clear compare the opalescence against a dark background, with that of the reference opalescence, through equal thicknesses of liquid.

After the oil has dissolved add an excess of the ethanol, as some oils precipitate on further additions of ethanol.

Express the results as follows:

'Solubility in X per cent (v/v) ethanol
= 1 volume in Y volumes, becoming opalescent in Z volumes'
(the words in italic type being added if appropriate).

If the solution is not entirely clear, record whether the opalescence is 'greater than', 'equal to', or 'less than' the reference opalescence.

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[*] Where difficulty is encountered in placing 1 ml in the tube, on account of the viscosity of the oil, the oil should be weighed and the volume calculated from the apparent density.

DETERMINATION OF RESIDUE ON EVAPORATION

a. Apparatus. Water bath with cover having holes of 70 mm diameter and provision for keeping the water level at approximately 50 mm below the cover throughout the test.

Evaporating basin of nominal capacity of 50 ml, made of heat-resisting glass inert towards essential oils, and conforming to the dimensions shown in Figure 1.

b. Procedure. Heat the evaporating basin on the vigorously boiling water bath for one hour, wipe the exterior, place it in a desiccator for 20 minutes and weigh it to the nearest milligramme. Weigh into the basin, to an accuracy of 1 mg, a suitable quantity of the oil (see Table 4), place it on the vigorously boiling water bath, screened from draughts, and heat for a continuous period of 5 hours. Remove the basin, wipe it and place it in a desiccator, and after 20 minutes weigh to the nearest milligramme.

c. Calculation. Residue on evaporation, per cent by weight = $\frac{100\ W_2}{W_1}$

where W_2 = weight, in grammes, of residue,
and W_1 = weight, in grammes, of sample taken.
Express the result to the first decimal place.

TABLE 4. WEIGHT OF OIL TO BE TAKEN FOR DETERMINATION OF RESIDUE ON EVAPORATION

Expected residue on evaporation	Weight of oil
per cent w/w	grammes
Below 5.0	4.8-5.2
5.0-8.0	2.8-3.2
Above 8.0	1.8-2.2

CHEMICAL METHODS

DETERMINATION OF ACID VALUE

NOTE. This method is not applicable to oil of wintergreen and oil of sweet birch.

a. Definition. The acid value of an essential oil is the weight of potassium hydroxide, in milligrammes, required to neutralize the free acids contained in 1 gramme of the oil.

b. Reagents. The reagents used shall be of a recognized analytical reagent quality. Distilled water or water of at least equal purity shall be used throughout.

Ethanol, 95 per cent (v/v).

Potassium hydroxide, approximately 0.1N ethanolic solution, standardized by titration with 0.1 N hydrochloric acid using phenolphthalein as indicator.

Phenolphthalein indicator, 0.2 per cent solution in ethanol, 60 per cent (v/v).

c. Procedure. Weigh into a flask, to an accuracy of 1 mg, not less than 2 g of the oil. Add 5 ml of the ethanol, freshly boiled and neutralized to phenolphthalein. Titrate with the 0.1 N ethanolic potassium hydroxide solution, using 0.2 ml of the phenolphthalein indicator.

d. Calculation. Acid value = $\frac{56.1 \times N \times V}{W}$

where V = volume, in millilitres, of the ethanolic potassium hydroxide solution required,

W = weight, in grammes, of oil taken,

and N = normality of the ethanolic potassium hydroxide solution.

Express the result to the first decimal place.

ESTERS

DETERMINATION OF ESTER VALUE

NOTE. This method is not applicable to oil of wintergreen and oil of sweet birch.

a. Definition. The ester value of an essential oil is the weight of potassium hydroxide, in milligrammes, required to neutralize the acids liberated by the hydrolysis of the esters present in 1 gramme of the oil.

b. Reagents. The reagents used shall be of a recognized analytical reagent quality. Distilled water or water of at least equal purity shall be used throughout.

Ethanol, 95 per cent (v/v).

Potassium hydroxide, approximately 0.5N ethanolic solution. Prepare by dissolving 33 g of potassium hydroxide in 1000 ml of the ethanol. Allow to stand and decant or filter the clear liquid.

Potassium hydroxide, 0.1N ethanolic solution.

Hydrochloric acid, 0.5N solution.

Phenolphthalein indicator, 0.2 per cent solution in ethanol, 60 per cent (v/v).

c. Apparatus. Flask, 250 ml capacity, made of chemically resistant glass and with a neck terminating in a ground socket*.

Reflux condenser, having a ground cone* for attachment to the flask.

d. Procedure. Weigh into a saponification flask, to an accuracy of 1 mg, a suitable quantity of the oil (see Table 5). Add 5 ml of the ethanol and neutralize with the 0.1N ethanolic potassium hydroxide solution, using the phenolphthalein indicator. Add to the neutralized solution 25.0 ml of the 0.5N ethanolic potassium hydroxide solution

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*B.S. 572, 'Interchangeable conical ground-glass joints'.

and boil the mixture under a reflux condenser for 1 hour*. Cool, add 20 ml of water and immediately titrate the excess of alkali with the 0.5N hydrochloric acid, using an additional 0.5 ml of the phenolphthalein indicator. Make a blank determination, following the same procedure but omitting the oil.

Ignore any reappearance of the pink colour on standing.

TABLE 5. WEIGHT OF OIL TO BE TAKEN FOR DETERMINATION OF ESTER VALUE

Expected ester value	Weight of oil
	grammes
Below 50	4.5-5.0
50-70	3.5-4.0
70-90	2.5-3.0
90-110	2.0-2.5
110-140	1.5-2.0
140-180	1.2-1.5
180-220	1.0-1.2
220-228	0.9-1.0

A. CALCULATION OF ESTER VALUE

$$\text{Ester value } E = \frac{28.05 \times (B - V)}{W}$$

where B = volume, in millilitres, of 0.5N hydrochloric acid required for the blank,

V = volume, in millilitres, of 0.5N hydrochloric acid required to neutralize the excess of alkali used for the hydrolysis,

and W = weight, in grammes, of oil taken.

B. CALCULATION OF CONTENT OF ESTERS

The percentage of esters present in the oil may be calculated from the formula (see Note) :

$$\text{Esters, per cent} = \frac{EM}{561}$$

where E = the observed ester value,

and M = the molecular weight of the ester.

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[*] With certain esters hydrolysis may be incomplete after 1 hour. In such case the period required for hydrolysis will be specified in the appropriate British Standard.

C. CALCULATION OF CONTENT OF COMBINED ALCOHOLS

The percentage of alcohols present as esters in the oil may be calculated from the formula (see Note) :

Combined alcohols, per cent $= \frac{EC}{561}$

where E = the observed ester value,

and C = the molecular weight of the alcohol.

NOTE. In the above formulae it is assumed that the alcohols are monohydri
and the acids monobasic.

DETERMINATION OF CARBONYL VALUE

Definition. The carbonyl value of an essential oil is the weight of potassium hydroxide, expressed in milligrammes, that is equivalent to the amount of hydroxylamine required to oximate the carbonyl compounds present in 1 gramme of the oil.

A. HYDROXYAMMONIUM CHLORIDE METHOD

This method is suitable for essential oils containing aldehydes other than citronellal and also for certain essential oils containing ketones.

a. Reagents. The reagents used shall be of a recognized analytical reagent quality. Distilled water or water of at least equal purity shall be used.

Ethanol, 95 per cent (v/v), free from aldehydes and ketones.

Hydrochloric acid, 0.5N solution.

Potassium hydroxide, approximately 0.5N ethanolic solution. Prepare by dissolving 33 g of potassium hydroxide in 1000 ml of the ethanol, allowing to stand, and decanting or filtering the clear liquid. Standardize against the hydrochloric acid, using the bromophenol blue indicator, and running the alkali into the acid.

Hydroxyammonium chloride solution. Dissolve 5 g of hydroxyammonium chloride in 95 ml of the ethanol; add 0.5 ml of the bromophenol blue indicator. Neutralize with the ethanolic potassium hydroxide solution until the solution is green when the liquid is observed in a thin layer, and red when the layer is thick. A lemon-yellow colour should be obtained by adding 0.05 ml of the hydrochloric acid to 20 ml of the solution, and a red colour by adding 0.05 ml of the ethanolic potassium hydroxide to another 20 ml of solution.

Bromophenol blue indicator. Dissolve, by warming, 0.2 g of bromophenol blue in 3 ml of 0.1N ethanolic potassium solution (prepared by diluting the 0.5N ethanolic solution with 4 volumes of the ethanol) and 10 ml of the ethanol. After cooling to room temperature make up to 100 ml with the ethanol.

b. Procedure. Weigh to an accuracy of 1 mg, into a 200 ml glass-stoppered conical flask, a suitable quantity of the oil (see Table 6), add 25 ml of the hydroxyammonium chloride solution. As will be indicated in the British Standard for the appropriate essential oil, either set aside for the time specified or titrate immediately with the ethanolic potassium hydroxide, taking care to avoid going beyond the greenish-yellow colour of the indicator, continuing the titration at intervals of not greater than 5 minutes until the bluish-green end point is reached and no further colour change takes place after a further 5 minutes.

If additional heating is required, this will be indicated in the appropriate British Standard.

TABLE 6

Expected carbonyl value	Weight of oil
	grammes
Below 50	9.0-10
50-100	5.0-6.0
100-200	2.5-3.0
200-300	1.7-2.0
300-400	1.2-1.4
400-500	1.0-1.1
over 500	0.8

B. FREE HYDROXYLAMINE METHOD

This method is suitable for essential oils containing aldehydes (including citronellal) and certain ketones, in the absence of substantial amounts of esters.

a. Reagents. The reagents used shall be of a recognized analytical reagent quality. Distilled water or water of at least equal purity shall be used.

Ethanol, 95 per cent (v/v), free from aldehydes and ketones.

Hydroxyammonium chloride solution. Dissolve 5 g of hydroxyammonium chloride in 95 ml of the ethanol; add 0.5 ml of the bromophenol blue indicator. Neutralize with the ethanolic potassium hydroxide solution until the solution is green when the liquid is observed in a thin layer and red when the layer is thick. A lemon-yellow colour should be obtained by adding 0.05 ml of the hydrochloric acid to 20 ml of the solution, and a red colour by adding 0.05 ml of the ethanolic potassium hydroxide to another 20 ml of solution. Filter if necessary.

Hydrochloric acid, 0.5N solution.

Potassium hydroxide, approximately 0.5N ethanolic solution. Prepare by dissolving 33 g of potassium hydroxide in 1000 ml of the ethanol, allowing to stand, and decanting or filtering the clear liquid. Standardize against the hydrochloric acid, using the bromophenol blue indicator, and running the alkali into the acid.

Bromophenol Blue indicator. Dissolve, by warming, 0.2 g of bromophenol blue in 3 ml of 0.1N ethanolic potassium hydroxide solution (prepared by diluting the 0.5N ethanolic solution with four volumes of the ethanol) and 10 ml of the ethanol. After cooling to room temperature make up to 100 ml with the ethanol.

b. Apparatus. Alkali-resistant glass flask of capacity 100 ml to 200 ml with a ground-glass joint* to which can be fitted a glass stopper or, when required, a glass tube at least 1 metre long and about 1 cm internal diameter, to serve as a reflux condenser.

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* B.S. 572, 'Interchangeable conical ground glass joints'.

c. Procedure. Weigh to an accuracy of 1 mg, into the alkali-resistant glass flask, a suitable quantity of the oil (see Table 7). Introduce, by means of a pipette, into a 200 ml conical flask 20 ml of the hydroxyammonium chloride solution, then add 10.0 ml of the ethanolic potassium hydroxide solution. Mix. Pour the liquid into the flask containing the essential oil and set aside the conical flask without washing. According to the instructions given in the British Standard for the particular essential oil being tested, let the contents of the first flask stand or boil under reflux for the time specified. When the latter procedure is followed, always cool rapidly before removing the reflux condenser. Neutralize with the hydrochloric acid till a greenish-yellow colour is observed. Transfer the liquid to the conical flask used to mix the reagent and the ethanolic potassium hydroxide solution and then pour half of it back into the first flask. Again neutralize the contents of one flask to a lemon-yellow colour, transfer to the other flask, mix and return one half to the emptied flask. Continue in this way until the point is reached at which the addition of two drops of the hydrochloric acid to the one flask causes no further change of colour by comparison with the other flask. Carry out a blank determination by the same procedure but omitting the essential oil.

TABLE 7

Expected carbonyl value	Weight of oil
	grammes
Below 25	5.5-6.0
25-50	2.8-3.0
50-100	1.4-1.5
100-200	0.7-0.8
200-300	0.5-0.6
300-400	0.4-0.5
400-500	0.3-0.4
over 500	0.3

C. CALCULATION OF CARBONYL VALUE

HYDROXYAMMONIUM CHLORIDE METHOD

$$\text{Carbonyl value } C = \frac{56.1 \times f \times V \times N}{W}$$

where V = volume, in millilitres, of N ethanolic potassium hydroxide used,

N = the normality of the ethanolic potassium hydroxide solution,

W = weight, in grammes, of oil taken,

and f = a correcting factor when the end-point of the reaction occurs at a pH different from that of N (or 0.5N) hydroxyammonium chloride.

NOTE. For Dimethyl Yellow and Methyl Orange f = 1.008 and for Bromophenol Blue f = 1.0.

FREE HYDROXYLAMINE METHOD

$$\text{Carbonyl value } C = \frac{28.05\ (V_1-V_2)}{W}$$

where V_1 = volume, in millilitres, of 0.5N hydrochloric acid required by the blank,

V_2 = volume, in millilitres, of 0.5N hydrochloric acid required by the sample,

and W = weight, in grammes, of oil taken.

D. CALCULATION OF CONTENT OF ALDEHYDE OR KETONE

HYDROXYAMMONIUM CHLORIDE AND FREE HYDROXYLAMINE METHODS

The percentage of carbonyl compounds, calculated in terms of a specific aldehyde or ketone, may be calculated from the formula :

$$\text{Per cent by weight of aldehyde or ketone} = \frac{C \times M}{561}$$

where C = the determined carbonyl value,

and M = molecular weight of the aldehyde or ketone.

b. General. The determination may be carried out by any well recognized method, but instruments employing the principle of the critical angle are most often used. 20°C is the standard temperature for the expression of results unless otherwise stated in the specification for a particular oil.

Diffuse white light may be used as an alternative to light from a sodium lamp for instruments fitted with an Amici compensator which has been adjusted, and the instrument calibrated for a wavelength of 589.3 nm*. Readings taken with white light are accurate only when a perfectly colourless and sharp line of demarcation is obtained between the dark and light fields.

c. Procedure. Carry out the determination at or as near as possible to the temperature specified.

d. Expression of results. Record the refractive index at 20°C as a number to four decimal places.

NOTE. The refractive index for the specified temperature may be calculated from that obtained at a temperature within 2 degC of the specified temperature by subtracting 0.0004 for each 1 degC the reading is taken below the specified temperature, or adding the same correction when the reading is taken above this temperature.

DETERMINATION OF FREEZING POINT

a. Apparatus. The apparatus recommended consists of a stout-walled glass test tube, 125 mm x 30 mm (inside measurements), fitted into a wide-mouthed jar or bottle of about 500 ml capacity by means of a bored cork; and an inner test tube, 100 mm x 21 mm, fitted into the larger tube also by means of a bored cork. The thermometer used should preferably comply with B.S. 1704+, being selected to be readable to 0.2 degC, and to have a diameter of about 5 to 6 mm and length of bulb between 15 mm and 20 mm.

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*1 nanometre (1 nm) = 10^{-9} metre = 1 millimicron (1 mμ).

+B.S. 1704, 'General purpose thermometers'.

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