INVESTIGATION OF THE VOLATILE OIL FROM CITRUS AURANTIFOLIA GROWING IN KENYA

BY

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INTRODUCTION

ABSTRACT

In the fruits of lime Citrus aurantifolia (Christm) swingle obtained from locally cultivated plants the volatile oil content was determined by steam distillation method. The highest yield of oil was 2.12%. The physical and chemical properties of the isolated oil were determined. The refractive index of the oil samples ranged from 1.480 to 1.481.

The study of the composition of oil was performed by thin layer chromatography. The oil was found to contain five main components of which 3 components, citral, linalool and 1-8 cineole were identified.

INTRODUCTION

The Citrus species belong to the family Rutaceae which embrances about 130 genera and approximately 1500 species. They belong to the tribe Citraceae and subtribed Citrinae. The subtribe includes six genera which are indigenous in an area extending from North East Australia and North Central China to Eastern Australia and New Caledonia (1)

The taxonomy of citrus species is often confused and complicated by the ease with which the plants hybridize by polyembryony, by mutation and by spontaneous production of auto tetraploid forms with their leaves and rinds (1)

The Citrus plants are cultivated mainly between 45° North and 35° South of equator (2) (3) Most of the commercial crop is grown in subtropical countries between sea level and 2,000 feet. On the equator they do not do well in altitudes above 6000 feet. The temperature should range between 15° and 38°C. An average rainfall of 80cm is essential in plants cultivated without irrigation. Humid tropics and high atmospheric humidity increased the incidences of pests and disease attacks. The trees grow over wide range of soils but light loamy soil of good fertility is preffered. The trees grow to a height of about 5 meters. The trees flower and bears fruits during the whole year.

The volatile oil occurs in various parts of the plants e.g in leaves, fruits and flowers. Citrus oils which are usually derived from the outer part of the fruits have become of considerable commercial importance. The production of citrus oils is one of the several subsidiary industries which have grown up in the main citrus growing regions of the world.

In citrus fruits the volatile oil occurs in the coloured part of the epicarp and is contained in oil glands of diameter ranging from 0.4 - 0.6 mm. Usually the oil glands are distributed irregularly in the outer coloured part (flavedo) of the mature and maturing fruits (3).

On the maturation of the fruits the albedo become elongated and branched forming an intricate network which gives the ripe peels its spongy texture. The oil from the peels can be obtained by simple expression or by steam distillation methods. To recover all the oil by distillation the peels are first broken up by mincing or grounding with due precautions to prevent loss of oil by spurting. The presence of water causes the rupture of cell wall to become easier. The expressed oils are of superior quality to distilled oil (4) (2).

The superiority of expressed oil appears to be due to the fact that there is virtually no contact between the oil and the inner part of the peel. Deterioration in odour is recorded

as the result of enzyme action in the finely divided albedo. The enzyme actions is more pronounced when the peel were finely ground. This causes an increased contact of albedo and water with the oil for any given length of time.

According to Guenther (5) the yield of oil varies from place to place. The oil yield from west Indies varied from 0.2, to 0.4%. The oil distilled in Ghana by Talalaj yielded 0.4% (6) The yield of oil in Ethiopia varies with altitudes. The oil content in trees grown at high altitudes was lower than those grown at lower altitudes (7).

The lime oil is composed of various components. In relation to the flavour the most important oil component is aldehyde which is present to the extent of 4%. The main oil component which amounts to about 90% is β-limonene. By fractional distillation, Guenther has separated several components of the oil (5). The aldehyde content of the oil in Ethiopia vary with altitudes. The aldehyde content of oil obtained from trees grown on lower slopes at 1000 metres above sea level was higher than that obtained from plants grown at high plains at 2000 - 3000 - metres. The oil from trees grown at high altitudes also showed higher - limonene content(7)

Lime oil is soluble in three parts of alcohol. According to Guenther (5) the refractive index of lime oils varies from 1.47 to 1.49. The refractive index of the oil distilled in Ghana varied from 1.4760 to 1.4782.

The oil was found to exhibit irritant action to the skin.(5) This is due to the presence of δ- limonene. Hence in perfumes and in liniments the oil is used in cutaneous irritant.

The pharmacological investigation of dried fruit preparation show an increase of blood pressure and the rate of blood circulation in dog's brain, kidney and coronary artery. In Guinea pig the preparation decreased heart rate and caused changes in ECG (9).

The lime oil is an important flavouring agent. It is the chief flavouring ingredient of carbonates, non alcoholic beverages of the ginger ale type as well as coca drinks and of ice-creams (10) (2). In perfumery industry the oil is used for the production of toilet waters which it imparts a refreshing odour.

The lime oil from citrus plants growing in Kenya has not been investigated yet. Since lime oil is of commercial importance it is necessary to have some data of the yield and composition of the oil from locally grown plants.

EXPERIMENTAL

METHOD

COLLECTION AND PREPARATION OF PLANT MATERIAL

Fresh lime fruits were obtained from the Nairobi market in December 1976. The fruits were divided into two parts. From one part the fruits were used without drying. From the other the peels were removed and divided into four parts. One part was dried at room temperature for 2 days, while the second part was dried at the same temperature for 7 days. The third part was dried in the oven at 40°C for 3 days, while the fourth part was used fresh.

Prior to distillation the plant material was thoroughly minced.

DETERMINATION OF THE VOLATILE OIL CONTENT

The determination of the oil content was carried out by steam distillation method described in British Pharmacopoea. Method number 1 for volatile oils lighter than water was applied (11).

Distillation time was 3 hours. 40g portions of the plant materials were used in each determination and the average results was calculated. Electrically heated 5l distilling flask was used. In order to get sufficient amount of oil for further investigation several distillations were carried out using larger amount of plant materials. The isolated oil was dried over

anhydrous Sodium sulphate and stored at low temperatures.

THIN LAYER CHROMATOGRAPHIC EXAMINATION OF THE OIL

Preliminary investigations were performed using microscope slides according to the method described by Stahl(12) Ten solvents were used in order to find the most efficient separating solvent systems. The following systems were applied:-

1. Hexane
2. Hexane: Ethyl acetate (85 : 15)
3. Benzene: 1% acetic acid (90 : 10)
4. Benzene: 96% acetic acid (90 : 10)
5. Benzene
6. Benzene: Chloroform (50 : 50)
7. Chloroform
8. Petroleum ether : Ether (80 : 20)
9. Hexane : Ethyl acetate (90 : 10)
10. Petroleum ether : Ether (90 : 10)

Hexane: Ethyl acetate (85: 15) was found to give the best sepration of components of the oil and therefore this system was used for the further study.

The spraying reagents used in preliminary investigations were anisaldehyde in sulphuric acid and Vanillin % in sulphuric acid prepared according to the method described by Stahl (13) Vanillin Sulphuric acid gave better results and hence it was used in all TLC examinations.

Glass plates 20 x 20 were used. The slurry was prepared using silica gel G, with water in the ratio of 1:2. The thickness of the layer was controlled by two bands of cellotapes. The layers were dried at room temperature and then activated at 110°C for 45 minutes. Prior to development the chromatographic tank was saturated with mobile solvent.

The volatile oil samples and reference substances were dissolved in toluene in the ratio of 1:50. The reference substances were obtained from I.C.I.P.E Micropipettes were used for spotting. The ascending TLC technique was applied after the method described by Stahl (14).

The solvent was allowed to develop to a distance of 15 cm. The spots were revealed by spraying with vanillin sulphuric acid reagent.

DETERMINATION OF PHYSICAL PROPERTIES OF THE OIL

The determination of refractive index was carried out according to the method described by Guenther (15) The determination of solubility was carried out according to the method described by Guenther (16) The solubility of the oil was determined using 70% alcohol.

The specific gravity and optical rotation were not determined due to lack of sufficient amount of oil.

R E S U L T S

The yield and physical properties of the oil are given in table 1. The results of the TLC separation of the oil is given in Fig. 1. The hRf values of the spots obtained are given in table 2.

Table 1

YIELD AND PHYSICAL PROPERTIES OF THE OIL

Plant material	Condition	Yield of oil per cent	Refractive index at 20°C	Solubility in 70% Alcohol
Peels	Dried at room temperature for 2 days.	0.75	1.480	3 parts
Peels	Dried at room temperature for 7 days.	0.40	1.480	3 parts
Peels	Dried at constant temperature of 40°C for 3 days	2.12	1.480	3 parts
Peels	fresh and undried	0.30	1.481	3 parts
Whole fruits	Fresh	0.10	-	-

Table 2

TLC EXAMINATION OF THE OIL

Spot	hRf value	Colour of the spot	Reference Substances.
A	28	dark tan	-
B	45	dark purple	linalool
C	48	dark green	citral
D	63	blue pink	1 - 8 cineole
E	76	Pink	-

DISCUSSION

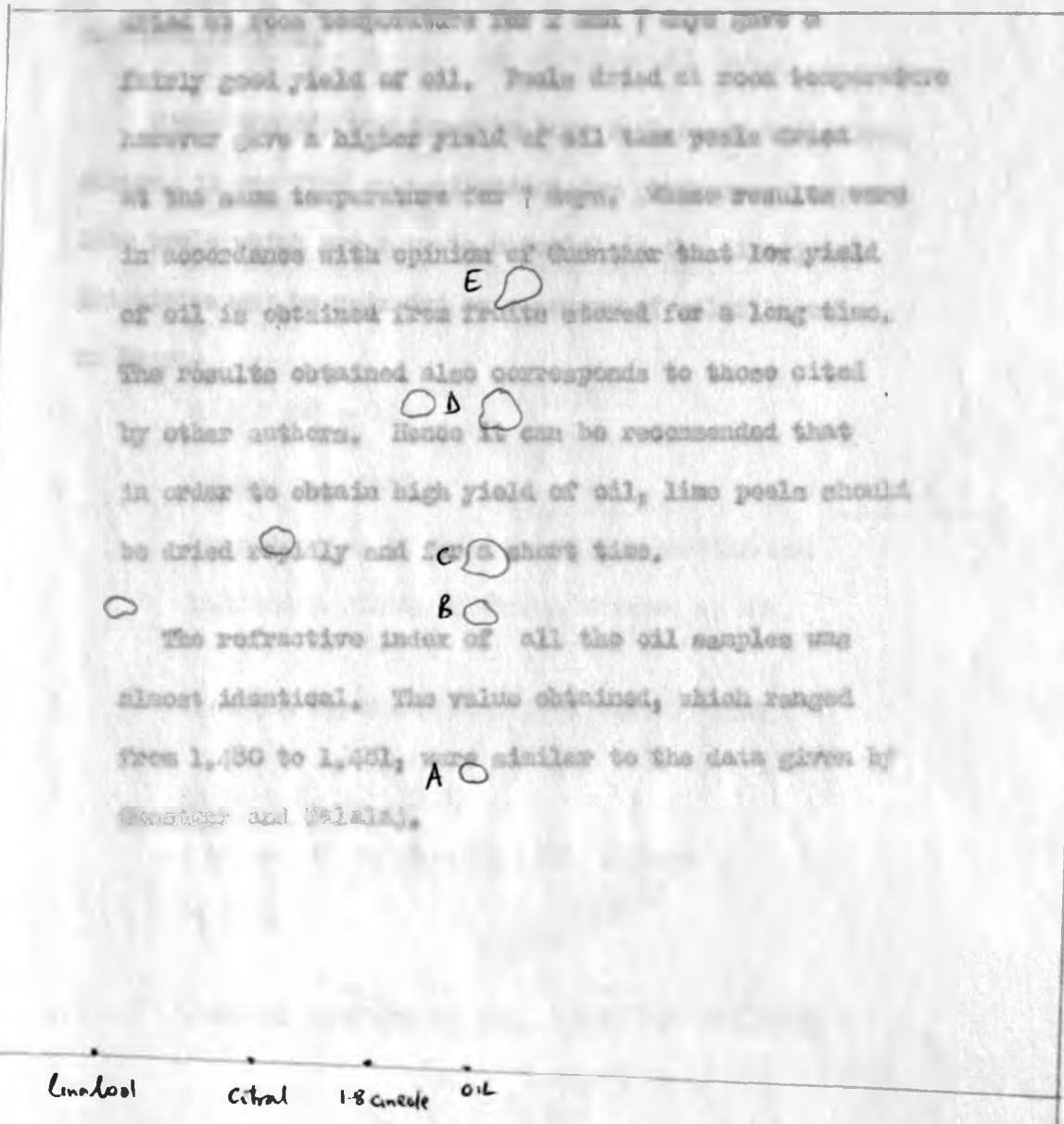
Fig 1

THIN LAYER CHROMATOGRAPHIC EXAMINATION OF THE LIME OIL

Developing Solvent: Hexane : Ethyl acetate (85: 15)

Spraying Agent: Vanillin 1%

Length of Run: 15 cm.



DISCUSSION

As seen from table 1 different yields of the oil were obtained from peels dried at different conditions. The low oil yields were obtained from the whole fruits and fresh undried peels. The plant material dried at 40°C for 3 days gave the highest yield of oil. Peels dried at room temperature for 2 and 7 days gave a fairly good yield of oil. Peels dried at room temperature however gave a higher yield of oil than peels dried at the same temperature for 7 days. These results were in accordance with opinion of Guenther that low yield of oil is obtained from fruits stored for a long time. The results obtained also corresponds to those cited by other authors. Hence it can be recommended that in order to obtain high yield of oil, lime peels should be dried rapidly and for a short time.

The refractive index of all the oil samples was almost identical. The value obtained, which ranged from 1.480 to 1.481, were similar to the data given by Guenther and Talalaj.

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DISCUSSION

As seen from fig. 1 the TLC examination shows five main components of the oil. The hRf value of the components were given in table 2. The hRf value of 3 components matches with those of reference substances. The results obtained show that the Kenyan lime oil contains citral, linalool and 1-8 cineole, however, further study is required to confirm these results by GLC and other chemical methods.

Since the oil was found to have a pleasant and strong odours, it may find an application for perfumery purpose. Lime peels which are a waste material in the lime juice industries may be regarded as a source of volatile oil in Kenya.

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