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Mani, Bijumon Kochukuttickal; Murthy, Vinuthaa; Boland, Martin; Yee, Kwang

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Analysis of constituents in different Fractions collected during distillation of Cardamom oil for flavour and fragrance applications

Biju K. Mani*, Vinuthaa Murthy, Martin Boland, Kwang Yee

School of Psychological and Clinical Sciences, Charles Darwin University – Darwin, Australia.

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ABSTRACT

Cardamom oil is used widely for flavouring in food, as medicine, in aromatherapy and as a taste enhancer in tobacco products as well as used in fragrance industry. Cardamom Oil is the essential oil of green cardamom fruit produced by steam distillation from the seeds after they've undergone a long drying and de-husking process. In the traditional distillation process, the oil collected on top of a separator half filled with water – as a whole. The main components contributing to flavour and fragrance properties of cardamom oil are alpha-pinene, eucalyptol, terpenes and a group of phenols occur at different stages and in different quantities during steam distillation. These components are released from the seeds during distillation based on its volatility. This study investigates the collection of cardamom oil in different fractions. Organoleptic as well as physical and chemical analysis including Gas Chromatograph and Mass Spectroscopy shows that there is a clear fractionation of the components during distillation, which helps the end user to choose the right fraction based on what the cardamom oil is used for.

INTRODUCTION

Essential oils have been known to mankind for many centuries. When the tomb of Egyptian Pharaohs of the 20th and 21st dynasties were opened in 1881, the scent of *Cymbopogon scheinantheus* grass was easily detectable (Washweton, 1906.) Oil of turpentine was well known to ancient Greeks. Almost 6000 years old traditional Indian system of medicine – Ayurveda, mentioned about distillation of spice oils in its recipes (Ajaikumar and Bharat, 2009). Today, this has developed into a large industry and many allied and value added industries have emerged from this ancient technology. (Bicchi and Sandra, 1987). Essential oils belong to the group of secondary metabolites from plants. Plants are metaphorically like a 'chemical factory', producing a wide range of complex compounds to promote growth and health (primary metabolites), some for defence and coexistence with the surrounding

eco-system (secondary metabolites) and some chemicals for reasons that are still unknown to science. Cardamom seeds and its essential oil or oil rich oleoresin are used widely for flavouring purpose in food, medicines, in aromatherapy and tobacco products as well as used in fragrance industry (Ajaikumar and Bharat, 2009). After saffron and vanilla, it is the third-most-expensive spice in the world (Winston, 2010).

It is the input to a range of products including food flavours, pharmaceutical goods (Joydeep *et al.*, 2015), basic ingredient for most of the perfumes, aromatherapy and industrial solvents. As perfumers, flavour manufacturers and other end users prefer different 'notes' (descriptors of scents that can be sensed upon the application, measured as top-middle-base notes, which denote groups of scents/taste which can be sensed/taste with respect to the time after the application of a perfume/flavour) (Reinarz, 2014), these notes are created carefully with knowledge of the evaporation process and intended use of the perfumery/flavour ingredients of the essential oil components. If the essential oils can be collected as different fractions, these fractions can give specific notes at its end use.

* Corresponding Author

E-mail: bijumani@hotmail.com

Table 1: Cardamom oil components (Ref. Parthasarathy *et al.*, 2008).

Main components			Trace components		
Components	Total oil %	Hydrocarbons	Acids	Alcohols and Phenols	Carbonyls
α -Pinene	1.5	α -Thujene	Acetic		3-Methyl bultanal
β -Pinene	0.2	Camphene	Propionic		2-Methyl bultanal
Sabinene	2.8	α -Terpinene	Butyric		Pentanal
Myrcene	1.6	cis-Ocimene	2-Menthyl butyric		Furfural
α -Phellandrene	0.2	trans-Ocimene	3-Menthyl butyric		8-Acetoxy carvotanacetone
Limonene	11.6	Toluene	Alcohols and Phenols		Cuminaldehyde
Eucalyptol	36.3	ρ -Dimethylstyrene	3-Menthyl butyric		Carvone
γ -Terpinene	0.7	Cyclosativene	ρ -Methyl-3-en-1-ol		Pinole
ρ -Cymene	0.1	α -Copaene	Perillyl alcohol		Terpiene-4-yl-acetate
Terpinolene	0.5	α -Ylangene	Cuminy alcohol		α -Terpinyl propionate
Linalool	3	γ -Cadinene	ρ -Cresol		Dilhydro- α -terpinyl acetate
Linalyl acetate	2.5	Δ -Cadinene	Thymol		
Terpinen-4-ol	0.9				
α -Terpineol	2.6				
α -Terpinyl acetate	31.3				
Citronellol	0.3				
Nerol	0.5				
Geraniol	0.5				
Methyl eugenol	0.2				
trans-Nerolidol	2.7				

Chemistry of the cardamom oil components is studied in detail by researchers (Kapoor *et al.*, 2011, Khan, 2010, Leela, 2008) and from various locations (Parthasarathy *et al.*, 2008) but no study is so far conducted for constituents in different fractions collected during distillation of cardamom oil for flavour and fragrance applications. Parthasarathy (Parthasarathy *et al.*, 2008) summarised main components and trace components of Cardamom oil as shown in Table 1.

The basic tests and analysis required for quality assurance of an essential oil starts with organoleptic analysis followed by simple physical and chemical analysis like Specific gravity, optical rotation and polarity. Gas Chromatograph (GC) and Mass Spectroscopy are common and are considered as the most important tools to analyse the constituents of essential oils. Gas chromatography-mass spectrometry (GC-MS) can add further improvements to essential oil analysis where broad categories of monoterpenes and varying isomers closely resembling each other in terms of chemical structure can be individually identified (Chin *et al.*, 2014). The GC-MS allows recognition of overlapping peaks on a GC by separating and identifying the constituents (Hunter, 2010).

A number of detectors can be used in chromatography and sensitivity has to be weighed off against the noise it produces. One of the most common is the flame ionization detector (FID), which is less sensitive than many specific detectors. FID detectors are mass rather than concentration sensitive and can detect most carbon containing substances, which makes them very suitable for the analysis of the majority of essential oils.

Raw materials for the study was tested and authenticated at Elixir Extracts, Kerala, India using analytical pharmacognosy tools (external morphology analysis, microscopy, volatile oil

content, volatile oil analysis, alcohol soluble extractive, water soluble extractive, ash percentage and moisture content)

MATERIALS AND METHODS

Production of essential oil of Cardamom

A stainless steel steam distillation vessel is cleaned and steam sparged for 12 hours to ensure it is contamination free. Freshly crushed suitable quantity (approximately 75% of the distillation vessel by volume) Cardamom seed is loaded on a perforated stainless steel sheet and steam is passed from the bottom. Condensed vapour with liberated essential oil molecules travel through a condenser and finally collected on water. Temperature of the loaded material is noted every 5 minutes and controlled below 75°C for 2 hours. This first fraction is drained out after 2 hours, labelled and preserved. Second and third fractions are collected for the next 12 hours and 8 hours each at 80°C and 85°C. The final fraction is collected for 4 hours or until the whole essential oil is liberated from the spent.

Preservation and sample collection

Anhydrous sodium sulphate is added to all the fractions immediately after collection (0.05% w/w) to remove moisture content. Sample is drawn to vials for analysis and sealed. Fractions are then transferred to air tight containers and kept in cool dry place under 25°C.

GC Analysis and confirmation using MS

To determine the chemical composition of the essential oil in Cardamom, GC-MS is used. Initially, the essential oil fractions is analysed using a gas chromatograph (Agilent 7890A)

equipped with an Agilent Flame Ionisation Detector (FID) and column (HP-5 5 % Phenyl Methyl Siloxan: Agilent 19091J-413, HP-5 5% Phenyl Methyl Siloxan, 325 °C: 30 m x 320 µm x 0.25 µm). The column is baked for 30 minutes at 280°C to minimise any chance of contamination and followed by oven programme method. Injection volume is 10 µl split with a split ratio of 1:10 and injection temperature was 250 °C. The temperature programmed between 40 and 250 °C in the following way: 50 °C for 1 min, 50-185 °C with 10 °C/min and 230 °C for 1 min.

The injections are repeated with a Gas Chromatograph coupled with Mass Spectroscope (Varian 431 integrated to Varian MS) using the same oven programme and flow properties. The auxiliary temperature is 230 °C and the mass spectrometer (MS) is operating in EI mode (70 eV) with a quadrupole temperature of 170 °C. The data obtained is analysed using the Chemstation integration programme (Leela, 2008) together with a NIST02 mass spectra search library. In this study, for the GC-MS we have used is a 30mtr capillary column for essential oil analysis, which has been found to be very effective (Rajapakse *et al.*, 1979) in analysis of volatiles with more than 150 ingredients.

Organoleptic Analysis

Smell, colour and taste are analysed using the traditional method. Smell strips are dipped in to the sample and air dried for 30 seconds and the smell is analysed. Colour is analysed against a light source and a small quantity is tasted and results noted. Solubility in water and alcohol (70% alcohol v/v) and volatility are analysed at room temperature using distilled water and ethyl alcohol. Texture also is observed (by checking the sample in test tube with naked eye for clarity and by using finger tips to check greasiness).

RESULTS AND DISCUSSION

Organoleptic Analysis

Organoleptic evaluation of steam distilled cardamom oil fractions was done and the characters were studied and summarised in Table 2. The solubility of the fractions (in solvents such as water and alcohol), volatility, colour, smell and texture are studied. The oils from initial fractions are clear and colourless to yellow when extracted freshly whereas later fractions are light

yellowish in colour, had strong aromatic and characteristic odour and bit greasy to touch. The oils are insoluble in water and soluble in organic solvents. All the fractions are lighter than water. These physical properties serve as a means of accessing the purity and quality of the volatile oils as well as their identification.

Identification of key compounds

The results obtained from the GC_MS were compared and the key compounds from each fraction were identified using overlay technique from the Chemstation32 software as well as NIST lib search of MS. The compounds identified in the 4 fractions are listed in Table 3. The GC chromatograms of all 4 fractions are shown in Figure1.

From GC analysis, of the first fraction, alpha-pinene has a retention time of 7.0 min and an area percentage of 71.5 and it is re confirmed using overlay and later with library in MS. Eucalyptol has a retention time of 13.04 min and an area percentage of 7.8. In the second fraction, area percentage of alpha-pinene is reduced to 42.64 at a retention time of 6.9 but area percentage of eucalyptol is increased to 37% at a retention time of 13.42 min. MS analysis supported this findings. In the 3rd fraction again the percentage of alpha pinene is further reduced to 1.35 at 6.9min and the eucalyptol increased to 66% at 13.62 min, which is again confirmed with MS analysis and data interpretation. Terpinyl acetate and geraniol occur at 15.5 min with approximately 2%. In the fourth fraction (where higher temperature is applied to the extraction vessel to liberate the last traces of essential oils) apart from small amount of alpha pinene, eucalyptol, geraniol terpinyl acetate, linalool and nirolidol are also detected.

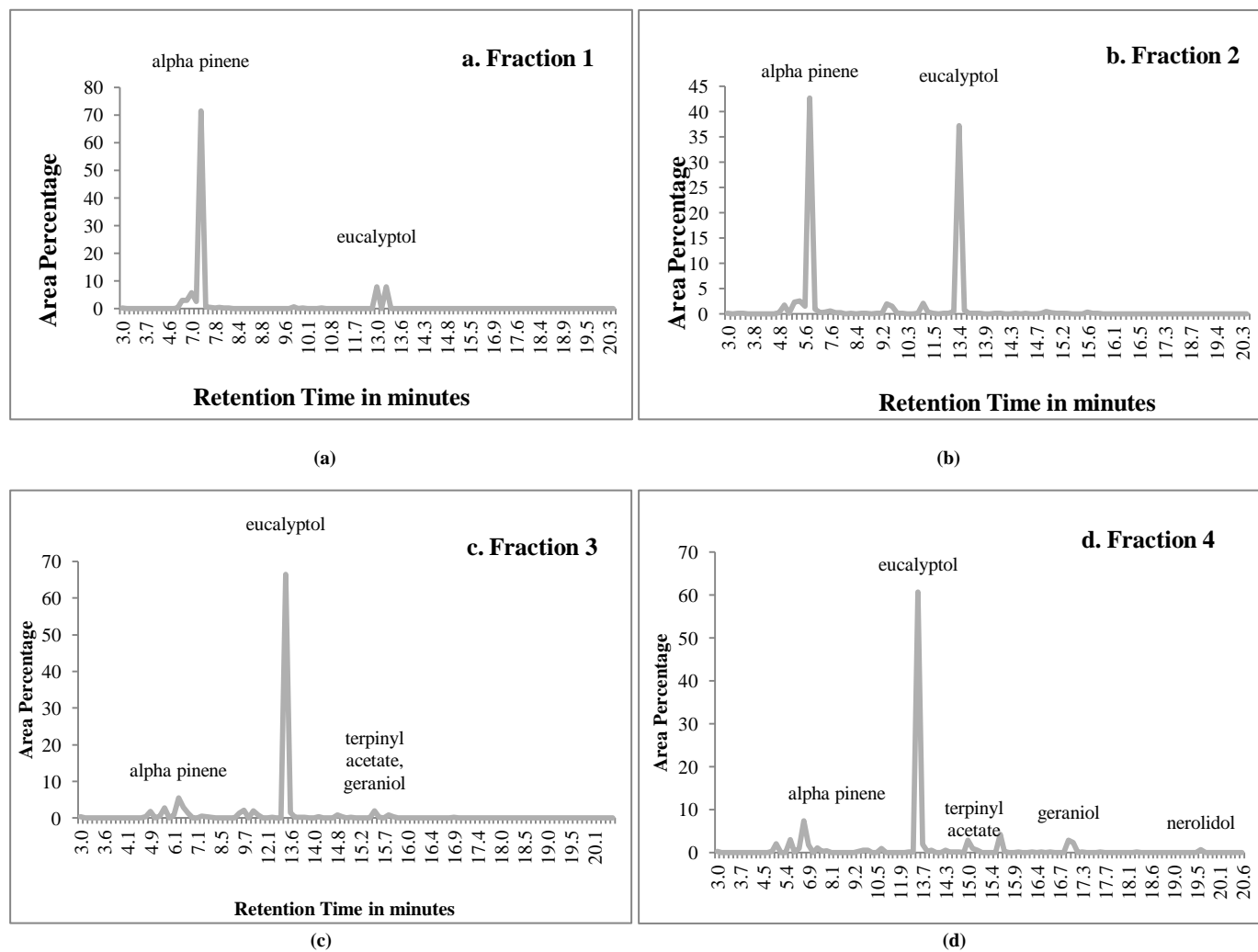
Quantity wise, alpha pinene, eucalyptol and α -terpinyl acetate are the major components in the cardamom volatile oil that contribute to the basic cardamom aroma. The major chemical constituents that impart sweet flavour to the oil are α -terpinyl acetate, geranyl acetate, nerol and α -terpineol; while eucalyptol imparts harsh camphory note (Zachariah, Pillai et al 2008). We find the third fraction has highest level of eucalyptol. α -terpinyl acetate content is recorded in 3rd and 4th fractions which has low alpha pinene content. Later fractions (3rd and 4th) contain geranyl acetate, linalool and nerol when compared to other fractions.

Table 2: Organoleptic properties of fractions.

Fraction	Smell	Colour	Taste	Solubility in Water	Solubility in Alcohol	Volatility	Texture
1	pinous, leafy	colourless	characteristic cardamom	insoluble	soluble	highly volatile	less turbid, clear
2	pinous, camphorous	colourless	characteristic cardamom	insoluble	soluble	medium volatile	less turbid, clear
3	camphorous, fruity	colourless to light yellow	characteristic cardamom	insoluble	soluble	medium volatile	clear
4	fruity, earthy	light yellow	characteristic cardamom	insoluble	soluble	volatile	Clear, slightly greasy

Table 3: Components of cardamom oil fractions with RT and area percentage.

Fraction No	Compound	Retention Time (Min)	Percentage
1	Alpha-pinene	7.0	71.59
	Eucalyptol	13.0	7.80
2	Alpha-pinene	6.9	42.64
	Eucalyptol	13.42	37.21
3	Alpha-pinene	6.9	1.34
	Eucalyptol	13.6	66.43
	Terpinyl acetate	15.5	2
	Geraniol	16.9	0.3
4	Alpha-pinene	6.5	7.4
	Eucalyptol	13.6	60.7
	Terpinyl acetate	15.1	2.9
	Linalool	15.5	1
	Geraniol	15.7	4.3
	Nerolidol	17	1.0

**Fig. 1:** Gas chromatogram of cardamom oil fractions. a) Fraction1, b) Fraction 2, c) Fraction 3, d) Fraction 4.

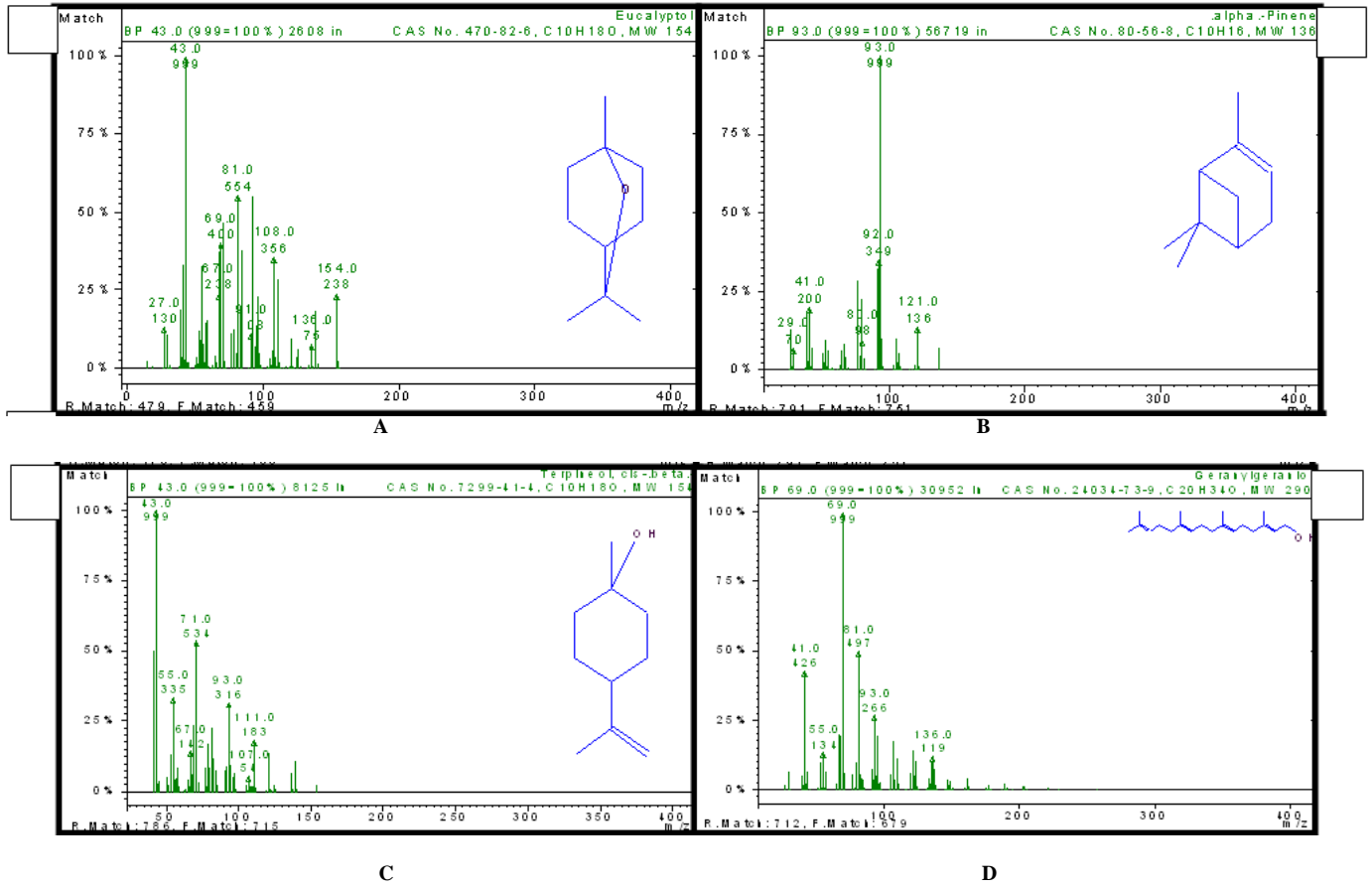


Fig. 2: Mass spectrograph of main compounds of cardamom oil: A – eucalyptol, B – alpha pinene, C – terpinyl acetate, D – geraniol.

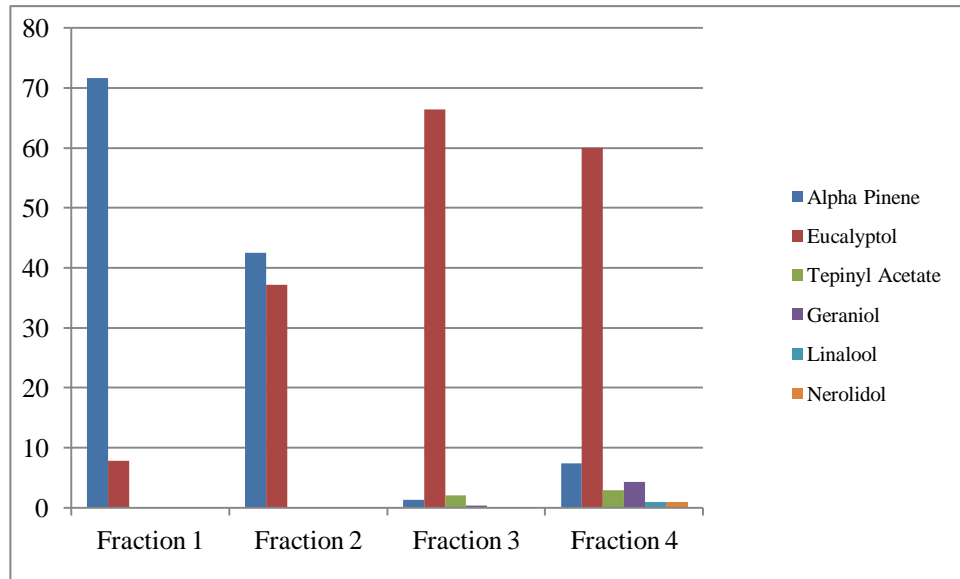


Fig. 3: Main ingredients of cardamom oil in each fraction.

CONCLUSION

From the GC-MS analysis it can be clearly seen that the two main components are alpha-pinene and eucalyptol. The other chemical constituents that impart the cardamom flavour appear in fraction 3 and 4. There is a clear fractionation of the components during distillation, which helps the end user to choose the right fraction based on what the cardamom oil is used for.

Phenols and alcohols, the main constituent in the fourth fraction are defined as compounds having molecules with one or more hydroxy group bonded to a benzene ring. Compounds like nerols, linalool, and geraniols are defined as phenols or alcohols. These oxidise easily and are partly the reason why essential oils darken after distillation. Phenols and alcohols in cardamom oil also darken on exposure to air and tend to oxidate (Hunter and Murray 2010). As the colour is the one of the main factor determining essential oil quality, separating the essential oils into few fractions conserves the quality of the oils. Hence we can state that it is ideal to collect and store fractions separately. One of the main end use of Cardamom oil is food flavouring and it is the main constituent of majority of the flavours used in pastry, biscuit, cakes and a lot of Ready to Cook (RTC) and Ready to Eat (RTE) semi processed food products. Essential oils lose small quantities of volatile compounds when baked or even stored at high temperatures (Dima and Dima, 2015). Hence, when the final food is baked or heated, there is a higher chance of loss of the components of the first fraction as the boiling point and flash point is very low (Dima and Dima, 2015). The heat withstanding ability is much better for the 4th fraction because it needs higher temperature to be released. Also, as the fourth fraction of the oil contains more phenols and alcohols and it tends to oxidise and change colour, it can be used to make oleoresins (a mixture of oil and resins) where colour is not a factor of quality or used in micro encapsulation (technique where each oil molecule can be coated with gum or modified starch) to prevent oxidation and also because it can withstand heat during cooking or baking

In perfumery and aromatherapy, the target temperature is very low (room temperature to body temperature) so it is ideal to use initial fractions (fraction 1 or 2) of essential oil rather than the last fractions (3 or 4) to get good results.

This study emphasises the importance of separated collection of Cardamom oil into four fractions during steam distillation of cardamom seeds. Study proved the need of establishment of collection of the Cardamom essential oil into four fractions based on the GC fingerprint, which will benefit in the quality of essential oil based on its end use.

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