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FATTYA ACID COMPOSITION AND FLAVOUR PROFILE ANALYSIS OF CARDAMOM ESSENTIAL OIL

M.D .SONTAKKE [1]., R.V. KALE [2]., V.R. CHAVAN AND G.S. RAUT [3]
[1]Department of Food Engineering, [2] Departmet of Food Process Technology,
[3]Department of Food Microbiology and Safety
MGM College of Food Technology, MGM Hills, Gandheli,
Aurangabad-431 007 (M.S.)-INDIA.
e-mail: ms.foodtec@gmail.com

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***Corresponding author**

ABSTRACT
Spices are food ingredients, popular in every cuisine they can be used in food products, due to their flavoring properties, taste, aroma, and color. In the present investigation the essential oil was extracted from cardamom by hydro-distillation using clevenger apparatus. The extracted essential oil were assessed for various physical and chemical characteristics. Results revealed that cardamom essential oil iodine value was found to be 90.56 (g/100g), acid value (8.7 mg KOH/g) and had lower peroxide value (7.5 g/kg). Fatty acid composition of cardamom essential oil were identified more than 40 compounds which represents 98.5 percent of all the compounds detected. The major compounds detected were *á*-Terpinyl acetate (37.55 percent), 1,8-Cineole (32.83 percent), Linalyl acetate (5.42 percent), Sabinene (4.99 percent), Linalool (4.44 percent), Myrcene (2.12), Limonene and *á*-Terpineol (1.88 percent) were investigated. Further, cardamom essential oil was also studied for fatty acid composition. It could be concluded that 1,8-cineole and *á*-Terpinyl acetate are the major component of cardamom essential oil known for its pleasant spicy aroma and taste. The cardamom essential oil can be used as a flavouring compound in formulation of food products.

INTRODUCTION

India ranks first in spices production, in the world, with an average production of 393190 Tons per annum in 2016-17. The estimated production of cardamom and cinnamon is 17990 and 150 Tonnes in the year 2016-17 respectively. The major producer states of small cardamom in India is Kerala has produced about 15650 Tons, Karnataka 1449 and Tamil Nadu 891 Tons in 2016-17. The average spices production by Maharashtra state has been estimated to about 393190 Tons of total spices production (Spice Board of India, 2018).

Spices are food ingredients, popular in every cuisine; they can be used in food products, not only because of their flavoring properties, taste, aroma, and color. The popular spices are cinnamon, cardamom, and ginger which are characterized by intense taste and aroma and have been used in human diet worldwide for a long time. Their flavoring application and impact on other product features are important (Aleksandra *et al*., 2017).

Cardamom (*Elettaria cardamomum* L.) is the most versatile spice known to the mankind as it enjoys a unique position in the international spices market. Green cardamom, also known as Malabar cardamom, small cardamom or Choti elaichi, is the true cardamom of commerce. The cardamom oil is found to be antimicrobial in nature and hence used in mouth fresheners and confectioneries. The volatile oils, and other high-value gastroprotective and antioxidant bioactives present in cardamom seeds mainly contribute to its characteristic aroma and role as a functional food, as well as its pharmaceutical and nutraceutical value (Hamzaa and Osman, 2012).

Cardamom essential oil has shown evidence of its antimicrobial, insecticidal, antioxidant and anti-inflammatory properties in different settings. Specifically, 1, 8-cineole (eucalyptol), the major active constituent found in Cardamom essential oil has been extensively studied for its anti-inflammatory activities (Han and Parker, 2017).

Essential oils are volatile secondary metabolites formed by aromatic plants and can generally be recognized by their characteristic odour. Their production is known to occur throughout the plant kingdom. Many epidermal cellular structures are capable of producing essential oils and there is a wide variety of chemical constituents (Martinelli *et al*., 2017).

Essential oils are used in a wide variety of consumer goods such as confectionery food products, soft drinks, and distilled alcoholic beverages. Their use as antioxidants and preservatives in food has been suggested, either incorporated into the foodstuffs and packaging material. Many essential oils have antioxidant and antimicrobial properties and, but their application as food preservatives requires a good knowledge of their properties, including the sensitivity of the target microorganisms, the specific mode of action, their antimicrobial potency, and the effect of food matrix components on their antimicrobial properties (Rios, 2016). The paper focuses on fattya acid composition and flavour profile analysis of cardamom essential oil

MATERIALS AND METHODS

The raw material used during the experiment was Cardamom

fruit of good quality was procured from local market of Parbhani.

Chemicals and Glassware

Chemicals of analytical grade and sufficient glassware required were available in the laboratory, Department of Food Chemistry and Nutrition, College of Food Technology. V.N.M.K.V. Parbhani.

Equipments and machineries

The equipments and machineries required during this course of study like electronic weighing balance, Clevenger apparatus, vernier calliper, hot air oven, Gas chromatography-mass spectrometry were available in the Department of Food Chemistry and Nutrition, College of Food Technology, VNMKV, Parbhani.

Methods

Extraction of essential oil

Essential oil was extracted by hydro distillation using Clevenger type apparatus. The raw cardamom seeds was coarsely ground using mortar and pestle were directly immersed in sufficient quantity of water into a extraction flask. The extraction flask was attached to the clevenger type apparatus and extraction process was carried out for 4-5h. The condensed mixture containing oil was separated in a Florentine flask due to their immiscibility and density difference that oil was collected in a pre weighed vial and the weight of oil collected was calculated. The traces of water in the oil were removed using anhydrous sodium sulphate, pure oil was stored at 4ºC in obscurity until the beginning of analysis (Al-Hashemi, 2014).

Flow sheet 1: Extraction of essential oil from spices by hydro distillation

Raw spices
↓
Cleaning
↓
Drying
↓
Grinding
↓
Hydro distillation (at 80-900C/ 3h)
↓
Separation (by separating funnel)
↓
Drying
(using anhydrous sodium sulpate)
↓
Packaging

Physical properties of extracted essential oil

The various physical properties of extracted essential oil were evaluated as per the method given by Barkatullah et al., 2012.

Colour

Colour characteristic of extracted essential oil was measured using visual observations.

Specific Gravity

The specific of essential oils was measured by using specific gravity bottle at 25ºC. The bottle was filled completely with oil and weighted. After cleaning, the bottle was filled completely with distilled water and weighed. The specific gravity was expressed in terms of ratio of oil to water.

Refractive index

The refractive index (n) of a substance is the ratio of velocity of light in vacuum to its velocity in the substance. It varied with the wave length of light used in its measurement. Clean the refractometer with alcohol & ether. A drop of oil or fat was placed on the prism. The prism is closed by the ground glass half of instrument. The depression screw is adjusted so that no color ting appears between the dark and illuminated halves. The dark line is adjusted exactly on the cross wires and refractive index is read on the scale.

Solubility

A known amount of solvent was put in a test tube then the oil was added in the test tube. A clear layer was observed indicate the solubility.

Viscosity

A Brookfield Viscometer Model DV-E was used to measure the viscosity of extracted oil. Viscosity was determined to at constant speed of 100 rpm and at constant temperature with a spindle number S-62 and it was expressed in terms of centipoise (cP).

Chemical characteristics of essential oil

The different chemical characteristics like acid value, iodine value, saponification value, peroxide value and ester value of extracted essential oil were carried out as per the method given by Kumar *et al*. (2014).

Acid Value

The acid value is the number of milligrams of KOH required to neutralize the free fatty acid present in 1g of fat. Hence acid value gives an indication of the age and quality of the fat. Free fatty acid percentage (as oleic) was determined by titrating oil in neutralized ethanol (95 percent) against NaOH solution (AOAC, 1990). The free fatty acid in oil is estimated by titrating it against KOH in presence of phenolphthalein indicator. The acid number is defined as 1 g of sample. However, the free fatty acid is expressed as oleic equivalents. 1 ml N/10 KOH = 0.028g Oleic acid. Acid value can be determined by the formula:

Acid value = % FFA x 1.99

Peroxide value

Peroxide value was evaluated according to AOCS Official Method Cd 8-53 (2003). Weigh out 5g of oil into a 500 ml conical flask, add 30 ml acetic acid chloroform mixture and dissolve the oil. Add 0.5 ml of saturated KI solution mix well and allow standing for 1 min. Add 30 ml of water, 3-4 drops of starch indicator and mixing well. Titrate against standard 0.01 N sodium thiosulphate with vigorous shaking to liberate all from chloroform layer until the blue color just disappears,

Treat the blank similarly in the absence of oil.

Peroxide value (meq O2/kg oil) =

$$\frac{(\text{Blank reading} - \text{Sample reading}) \times N \text{ of sodium thiosulphate}}{\text{Weight of sample}} \times 100$$

Iodine value

The iodine value of the sample was determined by AOAC methods was used. Accurately 0.4gm of the sample was weighed into a conical flask and 20ml of carbon tetra chloride was added to dissolve the oil. Then 25ml of iodine monochloride solution in glacial acetic (Wij's solution) was added to the flask using a safety pipette in fume chamber. Stopper was then inserted and the content of the flask was vigorously swirled. The flask was then placed in the dark for 2 hours and 30 minutes. At the end of this period, 20ml of 10% aqueous potassium iodide and 125ml of water were added using a measuring cylinder. The content was titrated with 0.1M sodium-thiosulphate solutions until the yellow color almost disappeared. Few drops of 1% starch indicator was added and the titration continued by adding thiosulphate drop wise until blue coloration disappeared after vigorous shaking. The same procedure was used for blank test and other samples. The iodine value is given by the expression: (AOAC, 1990).

Where,

Iodine value = g of iodine absorbed per 100 g of sample

B = Volume of titrant (ml) for blank

S = Volume of titrant (ml) for sample

N = Normality of Na2S2O3 (mol/1000 ml)

126.9 = MW of iodine (g/mol)

W = Sample mass (g)

$$\text{Iodine value} = \frac{(B - S) \times N \times 126.9}{W} \times 100$$

Saponification value

The saponification value is the number of milligram of KOH required to neutralize the fatty acids present as a result of the complete hydrolysis of 1g fat. Accurately weighed 2 g of oil into a 250 ml of conical flask, add 25 ml of alcoholic KOH and dissolve the oil completely. Connect air condenser to the flask and boil for about 30 min on a boiling water bath. Cool to room temperature, add 2 drops of phenolphthalein indicator and mix. Titrate against standard 0.5 N HCl until the pink color disappears. Treat blank similarly in absence of oil (A.O.A.C, 2000).

Ester value

Ester value is the number of milligram of potassium hydroxide (KOH) necessary to saponify estrs present in 1 g of the substances. Ester value of oil was calculated by using following relation:

Ester value = Saponification value – Acid value

Fatty acid composition of cardamom essential oils

Fatty acid composition of the essential oil was determined using Gas chromatography of FAMES (Fatty Acid Methyl Esters) with Flame Ionization Detector by AOCS Official Method Cd 14c-94 (2003). The oil (10–20 mg) was saponified for 1 hr with 1 ml of methanolic KOH (0.7 N) at 60 ºC, followed by neutralisation with 1 ml of methanolic HCl (0.7 N). The resulting free fatty acids were extracted in hexane and evaporated to dryness. The fatty acids were methylated using boron trifluoride (14% in methanol) and 0.2 ml benzene. The FAME was extracted in hexane, washed with water and evaporated to dryness. Fatty acid analysis was performed using a gas–liquid chromatograph (Shimadzu, GC-14B, Shimadzu Corporation, Japan) (Plate 6) fitted with a fused silica capillary column (BP 21: 30 m length, 0.30mm *i.e.*, 0.50 μm film thickness). The GC was equipped with a flame ionization detector, Clarity Lite 420 integrator and at isothermal conditions. The column temperature was set at 220ºC, the injector temperature at 230ºC and the detector temperature at 240ºC. Nitrogen gas was used as the carrier gas with a flow rate of 1 ml/min. Individual fatty acids in the oil were identified by comparison with the retention times of standard fatty acid methyl esters.

Flavour compound analysis of cardamom essential oil.

The flavouring compounds analysis of extracted essential oils was determined using Gas chromatography method (Adinew, 2014). GC-MS analysis of the essential oil sample was carried out on a PerkinElmer Auto System XL GC interfaced with Turbomass Quadrupole Mass Spectrometer fitted with an Equity-5 fused silica capillary column (60 m × 0.32 mm i.d., film thickness 0.25 μm). The oven temperature was programmed from 60-210 0C at 30C/min using helium as the carrier gas at 1.0 ml/min. the injector temperature was 2100C, injection volume 0.1 μl prepared in n-hexane (dilution 10%), split ratio 1:40. MS were taken at 70 eV with mass scan range of 40-450 amu and scan rate 1 sec with interscan delay 0.5 sec.

Identification of components

The component were identified on the basis of a Retention Index (RI), co-injuction with standards or known essential oil constituents. The relative amount of individual components was calculated based on the GC peak area (FID response) without using a correction factor.

RESULTS AND DISCUSSION

Physical properties of cardamom essential oil

Physical properties of essential oil indirectly inform about the quality of oil and provide the bases for suitability and utility of oils. Various physical characteristics of cardamom essential oil like specific gravity, refractive index, appearance and viscosity were determined and results are depicted in table 1.

Specific gravity is the ratio of the density of a respective substance to the density of water at 4ºC. The specific gravity values of oils are less than 1 for most of the oils except few containing oxygenated aromatic compounds (Barkatullah *et*

Table 1. Physical characteristics of Cardamom essential oil

Physical characteristics	Observations
Appearance	Slightly pale yellow Liquid
Solubility	Soluble in alcohol
Refractive index	1.465 ± 0.02
Specific gravity	0.923 ± 0.01
Viscosity (cP)	10.5 ± 0.2

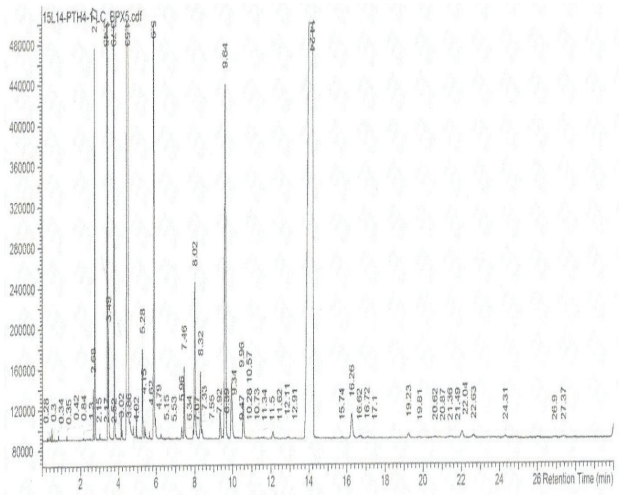

Figure 1: GC-MS peaks of principle components of cardamom essential oil

Table 2: Chemical composition of cardamom essential oil

Sr. No.	Chemical characteristics	Mean value
1	Saponification value (mg KOH/g)	209.0 ± 0.5
2	Iodine Value (g/100g)	90.56 ± 0.2
3	Acid value (mg KOH/g)	8.7 ± 0.02
4	Peroxide value (g/kg)	7.5 ± 0.01
5	Ester value (mg KOH/g)	200.3 ± 0.02

*Each value is the mean of three determinations

Table 3. Fatty acid composition of cardamom essential oil

Sr. No.	Fatty acids	Average value (mg/100g)
1.	Saturated	10.31
2.	Polyunsaturated	13.71
3.	Monounsaturated	6.1
4.	Trans fat	<0.1

al., 2012). From the data presented in table 1 showed that the specific gravity of cardamom essential oil was noted to be 0.923.

Refractive index of oils increases with increase in unsaturation and also chain length of fatty acids. Refractive index can be used to detect rancidity in edible oils, also provides info about purity of oils. Results summarized in above table showed that the refractive index of cardamom essential oil was found to be 1.465. Gende *et al*. (2008) reported similar results for specific gravity and refractive index.

Appearance of essential oils extracted from cardamom had colourless to slight pale yellow liquid. The results of physical properties of cardamom essential oil are in good agreement as reported by Ravindran and Madhusoodanan (2002). The viscosity of cardamom and cinnamon essential oil was noted to be 10.5 cP.

Chemical composition of cardamom essential oil

The chemical characteristics are also responsible for storability of oils besides quality of end product. The chemical properties such as acid value, iodine value, peroxide value, saponification value and ester value of essential oils extracted from spices were studied. The results of different chemical characteristics of cardamom and cinnamon essential oil are presented in table 2.

Data from table 2 revealed that the saponification value of cardamom essential oil was found to be 209.0. Iodine value is the measure of the proportion of unsaturated acids of fats oil present, the determination of iodine value measures the reaction of the double bonds with hydrogen. Higher iodine value indicates lower degree of saturation and vice versa (Mir *et al*., 2017). The iodine value of cardamom essential oil was found to be 90.56 (g/100g) had lowest acid value (8.7 mg KOH/g). The higher the acid value the lower is its storage quality and vice-versa. Essential oils are concentrated and contain several volatile compounds; often these are free fatty acids (Kumar, 2014).

Results demonstrated that the peroxide value of cardamom essential oil indicated lower peroxide value (7.5 g/kg). Peroxide value is the measure of deterioration of oil from oxidation and the freshness of lipid matrix. The rate of peroxidation differed from oil to oil (Kumar, 2014).

Results expressed for ester value indicated that cardamon essential oil had ester value 200.3 (mg KOH/g). The low ester value of the oil is concerned with the storage stability. The lower the ester value it can be kept for long period, which is due to the presence of short range acids (Mir *et al*., 2017).

Fatty acid composition of cardamom essential oil

Fatty acid profile with respects to saturated fat, polyunsaturated fat and monounsaturated fats of essential oil extracted from cardamom were determined by using gas chromatography mass spectroscopy. Results obtained for fatty acid profile are illustrated in table 3.

Data presented in table 3 revealed that the saturated fatty acids in cardamom essential oil was found to be 27.2 g/100g. Moreover, results noted that cardamom essential oil had (13.71 g/100g) polyunsaturated fats. Results shown for monounsaturated fats reported that cardamom essential oil found to have high monounsaturated fats (6.1 g/100g).

Flavour compound analysis of cardamom essential oil

The characteristic odour and flavour of cardamom oil can be determined by relative composition of components of volatile oil. The main factor that determines the quality of cardamom oil is the content and composition of volatile oil, which governs the odour and flavour (Zachariah, 2002). The essential oil extracted from cardamom were subjected to assessment of flavouring compounds by using Gas Chromatography Mass Spectrometry (GC-MS). Data related to flavouring compounds in cardamom essential oil are depicted in Table 4 and illustrated in Graph 1.

The essential oil profile of cardamom essential oil contained more than 40 compounds were identified represents 98.5 percent of all the compounds detected. It can be seen from the above table that the major compounds detected were *á*-Terpinyl acetate (37.55 percent), 1,8-Cineole (32.83 percent), Linalyl acetate (5.42 percent), Sabinene (4.99 percent), Linalool (4.44 percent), Myrcene (2.12), Limonene and *á*-Terpineol (1.88 percent) were investigated. It is evident that *á*-Terpinyl acetate and 1,8-Cineole was dominant components in cardamom essential oil. Results obtained are in close agreement with the findings reported by Gochev *et al*. (2012). Oil containing low amount of cineole but high content of terpinyl acetate are considered to be of superior quality for

Table 4: GC-MS flavour compound analysis of cardamom essential oil

Sr.No.	Compound name	Test method	Retention time (min)	Concentration (%)
1.	Isovaleral	GC-MS	0.51	0.02
2.	2-Methylbutyral	GC-MS	0.53	0.01
3.	±-Thujene	GC-MS	2.68	0.23
4.	Camphene	GC-MS	3.02	0.23
5.	Camphene	GC-MS	3.02	0.04
6.	±-Fenchene	GC-MS	3.02	0.04
7.	Sabinene	GC-MS	3.45	4.99
8.	²-Pinene	GC-MS	3.49	0.43
9.	Myrcene	GC-MS	3.79	2.12
10.	6-Methyl-5-hepten-2-one	GC-MS	3.86	0.03
11.	3-carene	GC-MS	4.02	0.01
12.	Octanal	GC-MS	4.15	0.17
13.	±-Terpinene	GC-MS	4.2	0.06
14.	1,8-Cineole	GC-MS	4.53	32.83
15.	Para-Cymene	GC-MS	4.53	0.1
16.	Limonene	GC-MS	3.76	2.61
17.	Cis-²-Ocimene	GC-MS	4.62	0.1
18.	trans-²-Ocimene	GC-MS	4.79	0.05
19.	³-Terpinene	GC-MS	4.95	0.16
20.	Cis-Linalool oxide	GC-MS	5.23	0.01
21.	Cis-sabinene hydrate	GC-MS	5.28	0.5
22.	Terpinplene	GC-MS	5.4	0.06
23.	Octanol	GC-MS	5.44	0.04
24.	6,7-Epoxymyrcene	GC-MS	5.64	0.04
25.	Linalool	GC-MS	5.9	4.44
26.	trans-sabinene hydrate	GC-MS	5.96	0.15
27.	Endo-Fenchol	GC-MS	6.24	0.03
28.	Borneol	GC-MS	7.33	0.11
29.	´-Terpineol	GC-MS	7.33	0.11
30.	Terpinen-4-ol	GC-MS	7.46	0.63
31.	±-Terpineol	GC-MS	8.02	1.88
32.	³-Terpineol	GC-MS	8.32	0.68
33.	trans-Piperitol	GC-MS	8.39	0.1
34.	Neral	GC-MS	9.34	0.34
35.	Linalyl acetate	GC-MS	9.64	5.42
36.	Geraniol	GC-MS	9.96	0.95
37.	Geranial	GC-MS	10.57	0.54
38.	Terpinen-4yl acetate	GC-MS	11.34	0.01
39.	´-Terpinyl acetate	GC-MS	12.11	0.11
40.	±-Terpinyl acetate	GC-MS	14.24	37.55
41.	Geranyl acetate	GC-MS	16.26	0.57

flavour applications. Mahmud, (2008) reported that 1,8-cineole as a major component of cardamom seed essential oil known for its pleasant spicy aroma and taste, can be used as flavourinds, fragrances and cosmetics. Similar results were obtained by Susheela, (2007) found sabinene, myrcene, *á*-pinene and linalool were identified in cardamom seed essential oil. According to Purseglove *et al*. (1981) the ratio of 1,8-cineole and *á*-Terpinyl acetate is fairly good index of purity and authenticity of cardamom volatile oil.

Leela *et al*. (2008) stated that 1,8-Cineole and *á*-Terpinyl acetate are the major components in cardamom essential oil and the basic cardamom aroma is produced by combination of these two. It was also reported that the major chemical constituents that that imparts sweet flavour to the oil are *á*-Terpinyl acetate, terpinyl acetate, nerol and *á*-terpineol while 1,8-cineole imparts harsh camphory aroma to the oil. The monoterpenes 1,8-cineole and camphor, have been shown to inhibit the germination and growth of competitors and thus acts as allelopathic agent (Zachariah, 2002).

It was found that the large number of flavouring compounds identified were present in trace amounts include Geraniol (0.95 percent), Geranyl acetate (0.57 percent), Cis-sabinene hydrate

(0.50 percent), Limonene (2.61 percent), Para-Cymene (0.10 percent), Cis-*â*-Ocimene (0.10 percent), *â*-Pinene (0.43 percent), *á*-Thujene (0.23 percent), Camphene (0.23 percent), *á*-Fenchene (0.04 percent), Octanal (0.17 percent), Neral (0.34 percent) and *ä*-Terpineol (0.11 percent) contributed about 6.5 percent of total compounds. Krishnamurthy and Sampathu (2002) postulated that the flavour of cardamom is entirely due to its volatile oil and the flavour strength is directly related to the quantity of oil present in seeds. Mysore oil has sweet and fruity-floral odour due to the lower amount of cineole and higher amount of terpinyl acetate, linalool and linalyl acetate (Lewis, 1973). Snoussi *et al*. (2015) reported that cardamom essential oils tested, 1,8-cineole which was the dominant compound found with a higher percentage also reported in our studies. Previous work, reported that the green cardamom was particularly rich in oxygenated monoterpenes (88.7%) with a dominance of *á*-terpinyl acetate (45.6%), 1,8-cineole

(26%). Additionally, several reports have shown that the basic green cardamom aroma from different geographic origin is a combination of 1,8-cineole and *á*-terpinyl acetate with different percentages. The composition of essential oils from a particular species of plant can differ between harvesting seasons and between geographical sources (Arras and Grella, 1992).

The chemical composition of cardamom oil varies considerably with variety, region and age of the product and also dependent on several factors and experimental conditions such as extraction method of essential oil and analytical conditions as reported by Husain and Ali, (2014).

The volatile oil with its characteristic aroma described as sweet, aromatic spicy, camphory. Cardamom oil found to be rich in oxygenated compounds, all of which were potential aroma compounds. While, many of the identified compounds alcohols, esters and aldehydes are commonly found in many spice oils, the dominance of the ether 1,8-cineole and the esters *á*-terpinyl and linalyl acetates in the composition, make the cardamom volatile oil a unique one (Raghavan, 2007). The bitterness compound present in cardamom may be due to *á*-terpinyl, present to the extent of about 0.8-2.7 percent in the oil (Zachariah, 2002).

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