GC- MS analysis of Volatile Flavour Compounds from Cardamom Flavoured peanut milk and their Biological Activities

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Abstract

Peanut milk was prepared by roasting method (roasting for 5 mins followed by soaking for 3 hrs) in CO 6 peanut variety. In the present study volatile bioactive compounds in control and cardamom flavoured peanut milk were identified by GC – MS analysis and identified the rapeutical bioactive compounds present in cardamom flavoured peanut milk. Good quality peanut was weighed and roasted for 5 mins followed by soaking for 3 hrs. Then it was grinded with kernel and water ratio 3:1 and filtered. Palm sugar, cardamom flavour, antioxidant, stabilizer was added and homogenized. The developed flavoured peanut milk was double pasteurized at 85º C for 30 mins. The prepared samples were freeze dried and used for the analysis of volatile compounds. Volatile compounds in the samples were analyzed by GC –MS analysis with suitable solvent. From the results it could be inferred that, compared to control cardamom flavoured peanut milk had linoleic acid ethyl ester, squalene, isoxazole, 5-chloro-4-(2-phenylethyl)-, 9-Octadecenoic acid bioactive compounds. The identified compounds had potent antioxidant, antimicrobial, anticancer and anti-flammatory effects.

Keywords: Flavoured peanut milk, Cardamom, volatile compounds, GC MS

Introduction

Peanut is an annual commercial cash crop and belongs to the fabaceae family or leguminacaea. It was a universal legume and most nutritious oil seed crops and grown well in subtropical and tropical climatic areas of Asia, America and Africa. Peanut was originated in South America in the area of Bolivia and Argentina.(Sharma and Bhatnagar-Mathur, 2006). Peanut is otherwise known as monkey nut, wonder nut, groundnut, poor men's cashew nut. (Madhusudhana, 2013).

World's largest peanut producer was China (45%) in peanut production. India and America holds an share of 16% and 5% of total peanut production. (Arya *et al.*, 2016). In developing countries like India though the production and productivity rate of peanut was high the consumption rate of peanut was low. Hence adequate research can be needed in these countries for processing the groundnut or developing new peanut related food products, it will be helpful for increasing the consumption of peanuts. (Yadachi *et al.*, 2012).

Due to some medical reasons like people having cow's milk allergy, phenylketonuria, cholesterol, lactose intolerance allergy, some of the lifestyle characteristics of the people like vegan diet people, conscious about antibiotic residues, growth hormone dairy and dairy

related products was avoided. In European countries nearly 15% of people avoiding dairy based products. (Jago, 2011).

Non dairy or plant based milk was easily digestible when compared to dairy milk and can be a suitable alternative for people with lactose intolerance. (Isanga and Zhang, 2008). Most of the children resist plain milk consumption due to bland taste and flavor, for those children flavored milk helps to increase consumption due to its flavour and its palatable form. It helps the children to meet out the nutritional target mainly calcium. It can be a suitable nutrient rich replacement for fruit and soft drinks. (Fayet-Moore, 2016).

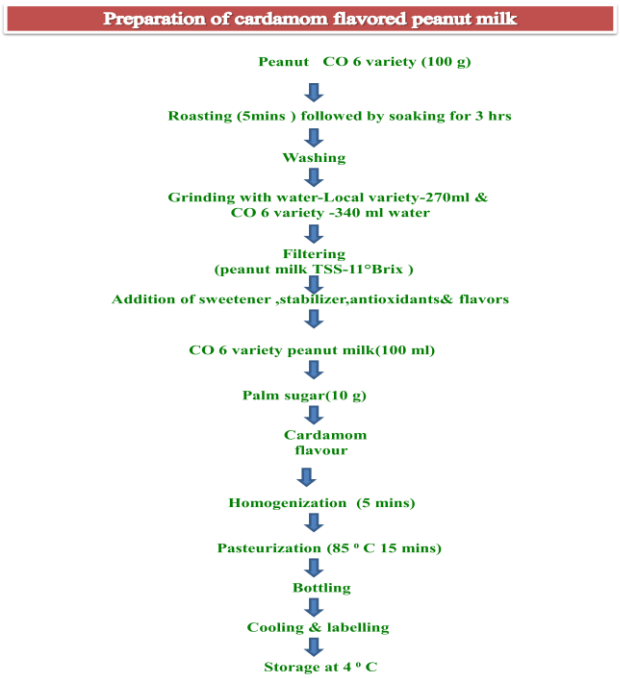
Flavour is defined as the combined perception of texture, taste and odour. In plants and animals during their metabolic process aromatic bioactive compounds were synthesized and responsible for the flavour of the food substances and also it can be modified by process. Reineccus, T. A *et al*., (2008).To find out the retention of volatile flavouring compounds present in the control and cardamom flavoured peanut milk using GC – MS analysis with suitable solvent extraction based on the literature the present study was carried out.

2. Materials and methods

2.1 Materials

TNAU CO 6 variety of peanut was procured from the Department of Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. Local peanut variety, palm sugar, cardadmom, food grade antioxidant (ascorbic acid powder) and xanthan gum were purchased from the local departmental stores of Madurai city.

2.2 Preparation of cardamom flavoured milk (Fig 1)



2.3 Identification of volatile compounds in flavoured peanut milk using GC-MS

2.3.1. Pre sample preparation

Standardized flavoured milk prepared from CO 6 peanut and local variety was kept at refrigerated condition for three days. Flavoured peanut milk was freeze dried for 6 – 7 hrs. Freeze dried powder was placed in the dessicator for one day and it was packed in a high-density polyethylene pack and stored at dark condition.

2.3.2. Sample preparation

2g of dried peanut milk sample was taken in a beaker and 20 ml of HPLC grade hexane was added. It was sonicated for 2 cycle (30 mins) and it was filtered using whatman's no.1 filter paper. Solvent in the sample was evaporated using solvent extractor and it was dissolved in a 2 ml HPLC grade hexane. It was filtered using membrane filter. Then the sample was transferred to the GCMS vial.

2.3.3. Gas chromatography – Mass Spectrometry analysis

Analysis of volatile compound using GC-MS was conducted using a QP2020 system (Shimadzu, Japan). 1 µL of sample extract was injected in hot split less mode (5 min split valve- delay time). Separation was performed in the non polar column (Rxi-5 Sil MS; 30 m length, 0.25 mm i.d and 0.25 µm film thickness). The oven temperature in the gas chromatography was programmed as follows: Initial temperature of 70ºC for 1 min and raised at a rate of 5 ºC/min to 225ºC with holding time of 3 min, then the temperature was raised to 280º C at a rate of 5º C/ min. Helium gas was used as a carrier gas. The programmed flow rate was 1 ml / min. Injector and MSD transfer line were held at 230º and 280ºC respectively. MSD conditions were as follows: electron- impact ionization voltage at the rate of 70 electron volts, scan range was 35 to 650 m/z at a rate of 2.74 scans/ s.

3.Result and discussions

Identification of components

Interpretation on mass spectrum GC – MS was done using the database of National Institute Standard and Technology (NIST), having more than 62,000 patterns. spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. Twenty five bioactive compounds were identified in control and cardamom flavoured peanut milk by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and area (%) of control peanut milk were presented in the Figure 1 - 2.

Figure 1. Chromotogram obtained from GCMS analysis of CO 6 variety control peanut milk

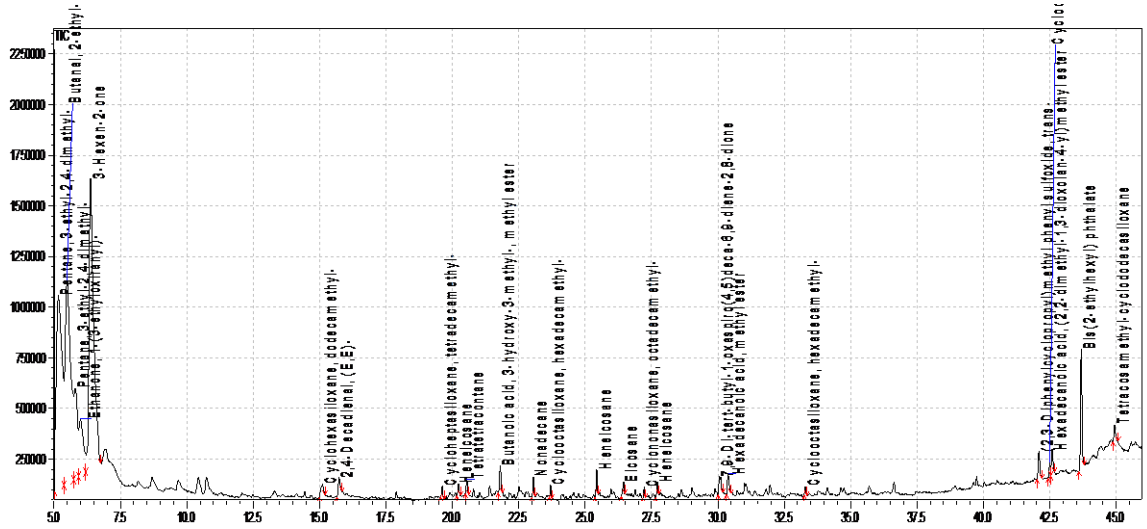
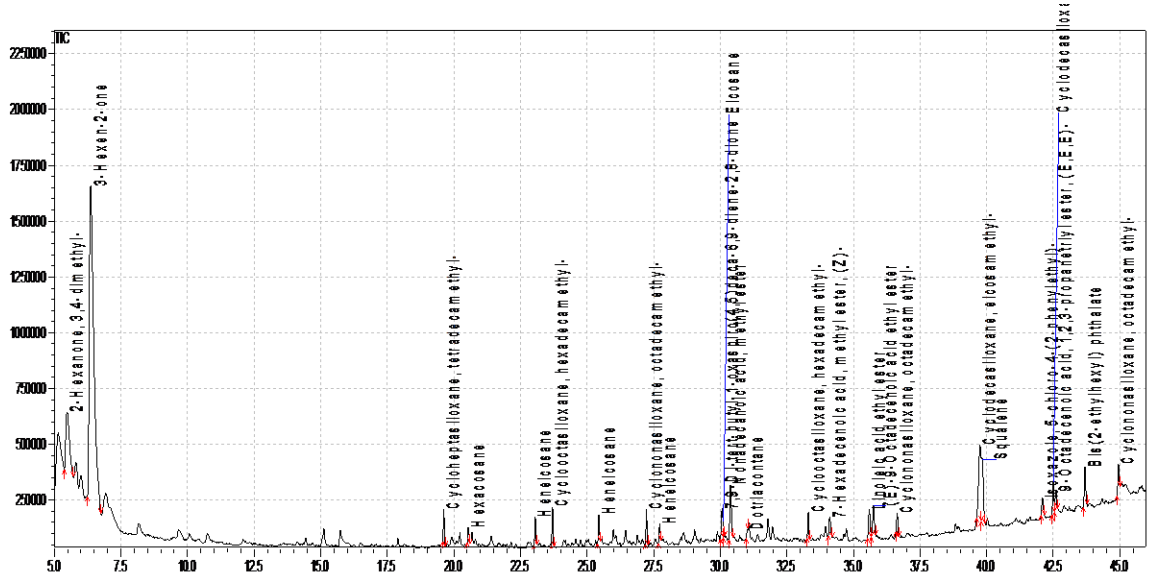


Figure 2. Chromotogram obtained from GCMS analysis of CO 6 variety cardamom flavoured peanut milk



The GC- MS Chromatogram of the volatile flavour constituents present in the samples are displayed in Figures 1- 2. The results showed that the presence of volatile flavor compounds in control and cardamom flavoured peanut milk samples were varied in number and peak area. The major twenty volatile flavour compounds present in control and cardamom flavoured peanut milk sample are presented in Tables 1-2. The study is concluded based upon the percentage of peak area.

Table 1 : Volatile compounds identified in CO 6 variety control peanut milk by GC- MS analysis

Peak#	R.Time	Area%	Name	Biological activity	Molecular formula	Molecular weight (g/mol)
1	5.161	23.63	Pentane, 3-ethyl-2,4-dimethyl-	Alkane	$C_{12}H_{26}$	128.25
2	5.486	23.56	Butanal, 2-ethyl-3-methyl-	Flavouring agent	$C_7H_{14}O$	116.20
3	5.800	6.54	Pentane, 3-ethyl-2,4-dimethyl-	Alkane	C_9H_{20}	128.25
4	5.990	5.10	Ethanone, 1-(3-ethyloxiranyl)-	Antioxidant, antimicrobial	$C_6H_{10}O_2$	114.14
5	6.375	27.85	3-Hexen-2-one	Flavouring agent	$C_6H_{10}O$	98.1430
6	15.113	0.69	Cyclohexasiloxane, dodecamethyl-	Skin care, cosmetics products, breast implantation, Antimicrobial	$C_{12}H_{36}O_6Si_6$	444.92
7	15.730	0.69	2,4-Decadienal, (E,E)-	Flavouring agent	$C_{10}H_{16}O$	152.23
8	19.619	0.27	Cycloheptasiloxane, tetradecamethyl-	Antimicrobial activity	$C_{14}H_{42}O_7Si_7$	519.07
9	20.226	0.29	Heneicosane	Antimicrobial	$C_{21}H_{44}$	296.5741
10	20.542	0.36	Tetratetracontane	Antioxidant and cytoprotective activities	$C_{44}H_{90}$	619.20
11	21.801	0.76	Butanoic acid, 3-hydroxy-3-methyl-, methyl es	Flavouring agent	$C_6H_{12}O_3$	132.11
12	23.047	0.47	Nonadecane	Antimicrobial	$C_{19}H_{40}$	268.518
13	23.697	0.26	Cyclooctasiloxane, hexadecamethyl-	Antimicrobial	$C_{16}H_{48}O_8Si_8$	593.2
14	25.436	0.58	Heneicosane	Antimicrobial	$C_{21}H_{44}$	296.5741
15	26.444	0.36	Eicosane	Antitumour activity against the human gastric SGC-7901 cell line antifungal .activity	$C_{20}H_{42}$	282.5475
16	27.226	0.23	Cyclononasiloxane, octadecamethyl-	Antimicrobial	$C_{18}H_{54}O_9Si_9$	667.4
17	27.717	0.32	Heneicosane	Antimicrobial	$C_{21}H_{44}$	296.5741
18	30.060	0.81	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene	To treat skin diseases, gonorrhea, migraine, intestinal parasites	C17H24O3	276.4
19	30.379	0.74	Hexadecanoic acid, methyl ester(lauric palmitic)	Antibacterial activity, Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic flavor, Hemolytic, Alphareductase inhibitor	$C_{17}H_{34}O_2$	270.45
20	33.297	0.22	Cyclooctasiloxane, hexadecamethyl-	Antimicrobial	$C_{16}H_{48}O_8Si_8$	593.2
21	42.088	0.96	(2,3-Diphenylcyclopropyl)methyl phenyl sulfo	Antitumour	$C_{22}H_{20}OS$	332.5
22	42.492	0.36	Cyclodecasiloxane, eicosamethyl-	hepato protective activity	$C_{20}H_{60}O_{10}Si_{10}$	741.5
23	42.587	0.78	Hexadecanoic acid, (2,2-	Antioxidant.	$C_{22}H_{42}O_4$	370.6

			dimethyl-1,3-dioxolan			
24	43.682	3.63	Bis(2-ethylhexyl) phthalate	Antibacterial and antifungal activity	$C_{24}H_{38}O_4$	394.6
25	44.943	0.56	Tetracosamethyl-cyclododecasiloxane	hepato protective activity, antispasmodic, antirheumatic, anti-soporific baths, insecticides for mosquito control, appetizing agent	$C_{24}H_{72}O_{12}Si_{12}$	889.8
		100.00				

In control peanut milk, Pentane, 3-ethyl-2,4-dimethyl-, Butanal, 2-ethyl-3-methyl-, 3-Hexen-2-one, Pentane, 3-ethyl-2,4-dimethyl-, Ethanone, 1-(3-ethyloxiranyl), Bis(2-ethylhexyl) phthalate, (2,3-Diphenylcyclopropyl)methyl phenyl sulfo, 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene, Hexadecanoic acid, methyl ester(lauric palmitic) were the major bioactive compounds based on percentage of peak area. (Table 1)

In cardamom flavoured peanut milk, 3-Hexen-2-one, 2-Hexanone, 3,4-dimethyl-, Cyclodecasiloxane, eicosamethyl-, Squalene, Bis(2-ethylhexyl) phthalate, 9-Octadecenoic acid, Nonadecanoic acid, methyl ester , 1,2,3-propanetriyl ester, 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien, Linoleic acid ethyl ester and Cyclononasiloxane, octadecamethyl were major bioactive compounds based on percentage of peak area. (Table 2).

Table 2. Volatile compounds identified in CO 6 variety control peanut milk by GC- MS analysis

Peak#	R.Time	Area%	Name	Biological activity	Molecular formula	Molecular weight (g/mol)
1	5.491	8.15	2-Hexanone, 3,4-dimethyl-	Flavouring agent	$C_8H_{16}O$	128.21
2	6.368	53.47	3-Hexen-2-one	Flavouring agent	$C_6H_{10}O$	98.14
3	19.622	1.30	Cycloheptasiloxane, tetradecamethyl-	Antimicrobial activity	$C_{14}H_{42}O_7Si_7$	519.07
4	20.542	0.70	Hexacosane	Antimicrobial activity	$C_{26}H_{54}$	366.71
5	23.051	1.22	Heneicosane	Antimicrobial	$C_{21}H_{44}$	296.5741
6	23.701	1.45	Cyclooctasiloxane, hexadecamethyl-	Antimicrobial	$C_{16}H_{48}O_8Si_8$	593.2
7	25.433	1.11	Heneicosane	Antimicrobial	$C_{21}H_{44}$	296.5741
8	27.230	1.32	Cyclononasiloxane, octadecamethyl-	Antimicrobial	$C_{18}H_{54}O_9Si_9$	667.4
9	27.720	0.78	Heneicosane	Antimicrobial	$C_{21}H_{44}$	296.5741
10	30.064	1.56	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien	To treat skin diseases, gonorrhea, migraine, intestinal parasites,	$C_{17}H_{24}O_3$	276.3707
11	30.115	0.38	Eicosane	Antifungal, antitumor antibacterial, larvicidal, antimicrobial, cytotoxic effects	$C_{20}H_{42}$	282.5475
12	30.379	3.25	Nonadecanoic acid, methyl ester	Antioxidant and Antimicrobial	$C_{20}H_{40}O_2$	312.5
13	31.005	0.08	Dotriacontane	Antimicrobial agent	$C_{32}H_{66}$	450.88
14	33.305	1.10	Cyclooctasiloxane, hexadecamethyl-	Antimicrobial	$C_{16}H_{48}O_8Si_8$	593.2

15	34.113	1.13	7-Hexadecenoic acid, methyl ester, (Z)-	Antioxidant, anti fungal, surfactant	$C_{17}H_{34}O$	268.4348
16	35.592	1.67	Linoleic acid ethyl ester	Hypocholesterolemic, Nematicide, Antiarthritic, Hepatoprotective Antiandrogenic , Hypocholesterolemic, 5-Alpha reductaseinhibitor Antihistaminic, Anticoronary, Insectifuge,Antieczemic,Antiacne	$C_{20}H_{36}O_2$	308.5
17	35.754	2.04	(E)-9-Octadecenoic acid ethyl ester	Cancer preventive, Insectifuge, Anti-inflammatory, Nematicide, Hepatoprotective, Antihistaminic, Anticane, Antiarthritic, Antieczemic	$C_{18}H_{32}O_2$	310.5
18	36.641	1.01	Cyclononasiloxane, octadecamethyl-	Antimicrobial	$C_{18}H_{54}O_9Si_9$	667.4
19	39.745	6.66	Cyclodecasiloxane, eicosamethyl-	hepato protective activity	$C_{20}H_{60}O_{10}Si_{10}$	741.5
20	39.835	4.73	Squalene	Antibacterial, Antioxidant, Antitumor, Anti-inflammatory, Antinociceptive, Potential antiplatelet components, Hypoglycemic, Hypolipidemic effects, Sedative action, Antihistaminic, Hepatoprotective activites Cancer preventing, Immunostimulant	$C_{30}H_{50}$	410.73
21	42.095	0.94	Isoxazole, 5-chloro-4-(2-phenylethyl)-	Antioxidant, aromatic compound	$C_{11}H_{10}ClNO$	664
22	42.499	1.49	Cyclodecasiloxane, eicosamethyl-	hepato protective activity	$C_{20}H_{60}O_{10}Si_{10}$	741.5
23	42.592	1.11	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (	Antagonism against bacteria	$C_{57}H_{104}O_6$	1059.5
24	43.688	2.07	Bis(2-ethylhexyl) phthalate	Antibacterial and antifungal activity	$C_{24}H_{38}O_4$	390.564
25	44.948	1.28	Cyclononasiloxane, octadecamethyl-	Antimicrobial	$C_{18}H_{54}O_9Si_9$	667.4

Some of the compounds identified in both control peanut milk and cardamom flavoured milk possessing biologically active properties like antibacterial, antioxidant, anti-inflammatory etc. The bioactive compounds such as 3-Hexen-2-one and Butanal, 2-ethyl-3-methyl- acting as flavouring agent in the peanut milk for beany flavor. Wang et al., (2012) reported that strong and exciting flavours of the dairy products could be attributed by alcohols, it can be formed by amino acid metabolism, aldehydes.

Ethanone, 1-(3-ethyloxiranyl)- bioactive compound was identified and had antioxidant and strong antimicrobial property, so it helps to destroy the food borne pathogens which helps in prevention from diseases. Bis (2-ethylhexyl) phthalate bioactive compound had antibacterial and antifungal activity it helps to prevent food borne disease. (2,3-Diphenylcyclopropyl) methyl phenyl sulfo bioactive compound was identified and it had antitumuor property it helps to prevent cancer. 7, 9-Di-tert-butyl-1- bioactive compound helps to treat skin diseases, gonorrhea, migraine, intestinal parasites. Hexadecanoic acid, methyl ester bioactive compound had antibacterial activity, antioxidant, hypocholesterolemic,

nematicide, pesticide, antiandrogenic flavor, hemolytic, alphareductase inhibitor property. Hexadecanoic acid, (2,2-dimethyl-1,3-dioxolan bioactive compound had antioxidant property.

The data reported that bioactive compounds in the cardamom flavoured peanut milk had much therapeutic and medicinal value. 3-Hexen-2-one and 2-Hexanone, 3,4-dimethyl- bioactive compound acts as a flavouring agent in cardamom flavoured peanut milk. Nonadecanoic acid, methyl ester bioactive compound had antioxidant and antimicrobial property it helps to prevent food borne pathogens related diseases. Cyclodecasiloxane, eicosamethyl-bioactive compound had hepato protective activity nature. (E)-9-Octadecenoic acid ethyl ester bioactive compound were identified and had cancer preventive, insectifuge, anti-inflammatory, nematicide, hepatoprotective, antihistaminic, hypocholesterolemic anticane, antiarthritic, antieczemic property.(Ponnamma *et al* ., 2012.,) In this present study, it was noticed that apparently, different volatile compounds were found in the control and cardamom flavoured peanut milk. These developments may be a function of double pasteurization processing culminating perhaps in either the release of volatiles already existing in the ingredients or degradation of amino acids, sugars and nucleotides.

The squalene is a phenolic compound it had several biological activities like antibacterial, antioxidant, antitumor, anti-inflammatory, antimicrobial, chemopreventive activity. (Rao C. V*et al*., (1998). Other beneficial activities like potential antiplatelet components, hypoglycemic, hypolipidemic effects, sedative action, antihistaminic, gastropreventive, hepatoprotective activities, cancer preventing, immunostimulant property. (Sunitha S *et al*., 2001.,) Cyclononasiloxane, octadecamethyl- bioactive compound had antimicrobial property.

Gaddaguti *et al.,* (2012) reported that Cyclohexasiloxane, dodecamethyl compound mostly used as an emollient, lubricant, deforming agent and conditioning agent. Ahsan *et al.*, (2017) reported that bioactive compounds like Eicosane and dibutyl phthalate had antifungal property it potentially against R. Solani AG-3 KX852461.Kawuri and Darmayasa, (2019) reported that Heneiconsane, Dodecane, Eicosane, and Cetene that have antimcrobial activity against bacteria and fungi.

Table 3 depicts the comparative analysis of volatile flavour compound present in control and cardamom flavoured peanut milk. The flavouring compounds 3-Hexen-2-one, Heneicosane, Eicosane, Bis(2-ethylhexyl) phthalate, 7-Hexadecenoic acid, methyl ester, (Z)-, Cyclodecasiloxane, eicosamethyl-, Cyclooctasiloxane, hexadecamethyl-, Cyclononasiloxane, octadecamethyl- was present in both control and cardamom flavoured peanut milk. The retention time and the percentage of peak area of the flavouring compounds retained were on par in control and cardamom flavoured peanut milk samples taken for the study.

Table 3 Comparative analysis of beneficial bioactive compounds in cardamom flavoured peanut milk compared to control peanut milk.

Volatile flavour compound present in control peanut milk					Volatile flavour compound present in cardamom flavoured peanut milk				
Peak#	**R.Time**	**Area%**	**Name**	**Molecular formula**	**Peak #**	**R.Time**	**Area %**	**Name**	**Molecular formula**
1.	6.368	53.47	3-Hexen-2-one	$C_6H_{10}O$	1	5.491	8.15	2-Hexanone, 3,4-dimethyl-	$C_8H_{16}O$
2	23.051	1.22	Heneicosane	$C_{21}H_{44}$	2.	30.379	3.25	Nonadecanoic acid, methyl ester	$C_{20}H_{40}O_2$
3.	30.115	0.38	Eicosane	$C_{20}H_{42}$	3.	31.005	0.08	Dotriacontane	$C_{32}H_{66}$
4.	43.688	2.07	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	4.	35.592	1.67	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$
5.	34.113	1.13	7-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{34}O$	5.	39.835	4.73	Squalene	$C_{30}H_{50}$
6.	42.499	1.49	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	6.	42.095	0.94	Isoxazole, 5-chloro-4-(2-phenylethyl)-	$C_{11}H_{10}ClNO$
7.	33.305	1.10	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	7.	42.592	1.11	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (	$C_{57}H_{104}O_6$

Conclusion

The volatile flavouring compounds present in the control and cardamom flavored peanut milk was analyzed by GC – MS. Compared to control peanut milk, cardamom flavoured peanut milk had additional volatile bioactive compounds like 2-Hexanone, 3,4-dimethyl, Nonadecanoic acid, methyl ester, Dotriacontane, Linoleic acid ethyl ester, Squalene, Isoxazole, 5-chloro-4-(2-phenylethyl)-, 9-Octadecenoic acid, 1,2,3-propanetriyl ester bioactive compounds it had more therapeutic and medicinal uses. Among these identified compounds, Linoleic acid ethyl ester, Squalene, Isoxazole, 5-chloro-4-(2-phenylethyl)-, 9-Octadecenoic acid has role in in antioxidant, antimicrobial, anticancer and antiflammatory effects.

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