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Research Paper

Use of GC-MS Combined with Resolution Methods to Characterize and to Compare the Essential oil components of Green and Bleached Cardamom

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Abstract: *Gas chromatography-mass spectrometry combined with iterative and non-iterative resolution methods was used to characterize and to compare the essential oil components of green cardamom and bleached cardamom. Green cardamom and Bleached cardamom essential oil components were extracted by microwave-assisted hydrodistillation and analyzed using gas chromatography-mass spectrometry. Thirty one and forty six components were identified by direct similarity searches for green cardamom and bleached cardamom, respectively. These numbers were extended to fifty five and seventy components, respectively with the help of multivariate curve resolution methods. Morphological score and subspace comparison were used for chemical rank determination of GC-MS data. Multivariate curve resolution-alternative least squares as an iterative method was used for resolving the overlapped and embedded peaks. Comparison of the results of green cardamom and bleached cardamom showed that their volatile components are different from chemical components and relative percentages points of view. Major constituents in green cardamom are 1,8-cineol (47.18%) , alpha-terpinyl acetate (14.33%) , linalool (6.28%) , terpineol (4.94%) l-4-terpineol (2.48%) and in bleached cardamom are 1,8-cineol (34.12%) ,alpha-terpineol (26.91%) , alpha-terpinyl acetate (21.04%), linalool (6.39%) and l-4-terpineol (1.89%). In spite of different cultivation conditions, there are 33components which are common between the two types of cardamom.*

Keywords: Gas Chromatography-Mass Spectroscopy, Green Cardamom, Bleached Cardamom, Chemometric, Multivariate Curve Resolution, 1,8-cineol.

Introduction

Green cardamom or Small cardamom, known as the 'queen of spices', which belongs to the family of Zingiberaceae, is a rich spice. It is one of the highly prized spices of the world and is the third most expensive spice after saffron and vanilla. The original home of this precious spice is the mountains of the south-western parts of the Indian Peninsula. As early as the 4th century BC, cardamom was used in India as a medicinal herb and was an article of Greek and Roman trade [1]. Cardamom grows abundantly in forests at 760–1500 meter above sea level, preferring shady locations and rich, moist, well-drained soil. It is widely cultivated in India, southern Asia, Indonesia and Guatemala. The fruit is an ovoid, three-celled, dehiscent capsule containing many seeds, which are covered by an aril. During drying, it is said to lose three- quarters of its weight. Cardamom is used as an aromatic, carminative and stimulant. The seeds have a warm, slightly pungent aromatic flavor. It is used mainly as a flavoring agent in tea and food preparations. Cardamom oil is a precious ingredient in food preparations, perfumery, health foods, medicine and beverages. Cardamom is also used internally for indigestion, nausea, vomiting and pulmonary disease with copious phlegm and also as a laxative and flatulence to prevent stomach pain. The seeds are also chewed to sweeten the breath and taken to detoxify caffeine in people drinking excessive amounts of coffee [2]. Bleached

cardamom is creamy white or golden yellow in color. Bleaching can be done either with dried cardamom capsules or freshly harvested capsules as starting material [3]. In general, bleaching of dried capsules lead to loss of volatile oil probably because the bleaching process makes the husk brittle. However, bleached cardamom has white appearance and is resistant to weevil infestation due to sulfur dioxide content [4,5].

Gas chromatography–mass spectrometry (GC–MS) is one of the most successful techniques for the determination of the components of essential oils [6-9]. Second order instruments involving separation are ideally suited for the analysis of complex samples and are frequently used as powerful tools for chemical analysis. For GC–MS technique, much more components are qualitatively and quantitatively analyzed, but their identifications are performed only through the direct similarity searches in the MS databases attached to the GC–MS instruments. Even under the best experimental conditions, the probability of overlapped peak in chromatographic separations can become quite severe, especially for highly complex samples.

This is due to the existence of the background, baseline offset, and some overlapping/embedded peaks. These problems can result in a wrong similarity match in the MS library and therefore, true determination of the components cannot be achieved. In these cases, resolution and afterwards quantification of the target compounds becomes a goal. Fortunately, with the development of chemometric resolution techniques, the extraction of required information about the components in a complex mixture has become possible. Multivariate curve resolution (MCR) methods have been used for the analysis of unresolved peaks in chromatographic separations coupled to multichannel detection such as high performance liquid chromatography–diode array detector (HPLC–DAD), liquid chromatography–mass spectrometry (LC–MS), and GC–MS [10].

In this work, Microwave-assisted hydrodistillation (MAHD) method has been used to extract the volatile components of green cardamom and bleached cardamom essential oils, microwave assisted methods have been used increasingly in the last few years, especially for extraction. GC-MS has been used for the determination of essential oil's components. Due to the complexity of essential oils and existence of overlapping peaks, chemometric resolution techniques was used for resolving the co-eluted GC-MS peak clusters.

Material and Methods

Indian Green cardamom and bleached cardamom were purchased. Normal hexane and anhydrous sodium sulfate with purity higher than 99% were purchased from Merck (Germany).

Extraction of volatile components of green cardamom and bleached cardamom

100 g of beaten cardamom submerged in water in a round bottom flask and was hydrodistilled for 45 minutes using an adapted microwave distillation apparatus at 700 W. This instrument consisted of a microwave oven connected to a clevenger-type apparatus as illustrated in Figure 1.

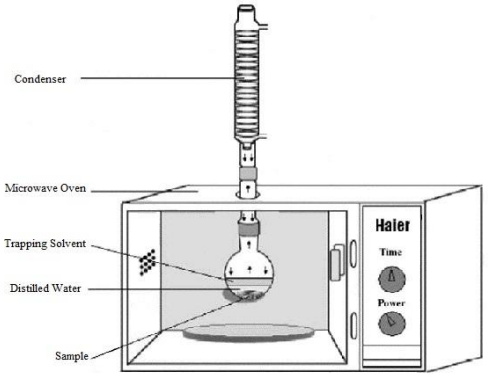

Figure 1: Adapted microwave distillation apparatus

The produced essential oil was collected in a dark glass bottle and it was dried using anhydrous sodium sulfate, then it was stored at 4 ˚C until GC-MS analysis.

Gas chromatography-mass spectrometry analysis (GC-MS)

GC-MS analysis were performed with the use of HP-Agilent 6890 GC that has been coupled with a HP-Agilent 5973 mass selective detector and was equipped with RTX-5 capillary fused silica column (30 m, 0.25 mm i.d. and 0.25-µm film thickness). Temperature programming has been preformed under following condition: the oven temperature was held at 50 ˚C for 5 min, then programmed at 4 ˚C min^{-1} to 250 ˚C, held for 5 min. Other operating conditions were as follows: carrier gas, He (99.99 %), injector type, splitless. In MS, voltage and ionization source temperature were 70 eV and 220 ˚C, respectively.

Identification

The essential components were identified by calculating their Kovats retention indices (RIs) and comparing them and mass spectra with RIs and mass spectra of standard compounds stored in NIST mass spectral database.

Data analysis and software requirements

MCRC software [11] was used for preprocessing, chemical rank determination and local rank analysis. preprocessing methods such as baseline correction, denoising and smoothing have been done on input data in order to obtain more accurate results, in

addition chemical rank determination and local rank analysis have been performed prior to resolution step. In The resolution step MCR-ALS has been used to extract the pure mass spectrum and chromatographic profile of each component from the original GC-MS data matrix. Finally, the essence of each pure component can be determined by comparing its resolved mass spectrum with those of mass libraries and the relative percentage of each component can be calculated [12]. A software G1701DA MSD ChemStation version D.00.01 was used to collect data and conversion to ASCII format. Programs of the chemometric resolution methods were coded in MATLAB R2009a by authors. library searches and spectral matching of the resolved pure components were conducted using the NIST MS database.

Theory

Microwave-assisted hydrodistillation (MAHD)

With today's emphasis on speed and efficiency in the analytical chemistry laboratory, any technique that will improve speed and efficiency of solvent extraction is an important one. Since 1985, applications of microwave heating for the extraction of compounds from many sample matrices have been in use. Microwave-assisted extraction is the process of heating solvents in the contact with a sample with microwave energy to partition compounds of analytical from the sample matrix into the solvent [13]. The optimization of microwave-assisted extraction conditions has been studied in several applications. The efficiency of the process is directly related to the operation conditions selected. Special attention should be given to usually studied parameters that may influence the performance of MAE such as solvent composition, solvent-to-feed ratio, extraction temperature and time, microwave power, and the characteristics of the matrix including its water content. Comprehension of the effects and interactions of these factors on the microwave-assisted extraction process is significant [14].

Multivariate curve resolution (MCR)

The resolution of a multicomponent system involves the description of the variation of measurements as an additive model of the contributions of their pure constituents. To do so, relevant and sufficiently informative experimental data are needed. These data can be obtained by analyzing a sample with a hyphenated technique (e.g., GC-MS or HPLC-DAD) [15]. In the resolution of any multicomponent system, the main goal is to transform the raw experimental measurements into useful information. Resolution methods are powerful approaches that do not require a lot of prior information because neither the number nor the nature of the pure components in a system need to be known beforehand. Any information available about the system may be used, but it is not required.

All resolution methods mathematically decompose a instrumental response of mixtures into the contributions linked to each of the pure components in the system. This global response is organized into a matrix D containing raw measurements about all of the components present in the data set. Resolution methods allow for the decomposition of the initial mixture data matrix D into the product of two data matrices C and S^T, each of them containing the pure response profiles of the *n* mixture or process components associated with the row and the column directions of the initial data matrix, respectively. In matrix notation, the expression for all resolution methods is:

$D = CS^T + E$

Where D (r×c) is the original data matrix, C (r×n) and S^T (n×c) are the matrices containing the pure-component profiles related to the data variation in the row direction and in the column direction, respectively, and E (r×c) is the error matrix. The variables r and c represent the number of rows and the number of columns of the original data matrix, respectively, and n is the number of chemical components in the mixture or process. C and S^T often refer to concentration profiles and spectra [15].

Results and Discussion

Qualitative analysis of green and bleached cardamom essential oil

TICs of green cardamom and bleached cardamom are shown in Figure 2 (a) and (b). The appearance of TICs demonstrate the complexity of these essential oils. It seems that some peaks are overlapped. Therefore, the overlapped and embedded peaks must be resolved into pure chromatographic profiles and mass spectra for the accurate qualitative and quantitative analysis. In this work, TIC of green cardamom and bleached cardamom were divided to 53 and 67 peak clusters, respectively using zero component regions along elution sequence for the essential oil. According to the morphological score method [16] Some of these sub-matrices are single component peaks. These peaks can be easily identified and quantified by direct library searches and peak integration in ChemStation software. However, to have a more reliable results, single component peak clusters were pretreated and decomposed using PCA method and chromatograms, and mass spectra were obtained from corresponding scores and loadings. It should be noted that a same method like the ChemStation software was used for peak integration.

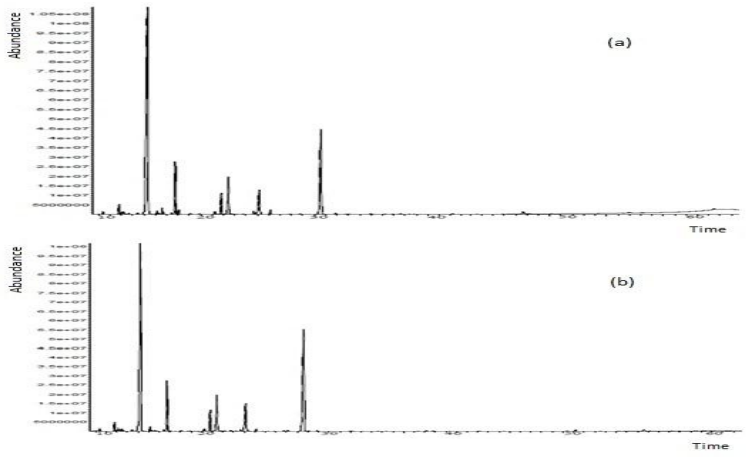

Figure 2: Total ion chromatograms (TICs) for (a) Green cardamom (b) Bleached cardamom

Therefore, for single component peaks, simple peak integration can give the peak area for the desired component. In addition, due to the absence of standards for resolved components, so, relative quantitative information can be obtained. This is a common strategy used in essential oil analysis. The obtained results were much better than those of ChemStation from the match factor (MF) and percentage of each component points of views. In order to illustrate the resolution procedure, as an example, one peak cluster from TIC of green cardamom is selected from retention times of 12.10-12.85 min. The TIC of this peak cluster is shown in Figure 3. This peak cluster was extracted using MSD ChemStation software and was changed to ASCII format that was compatible with MATLAB software. Direct library search for this peak cluster before performing resolution procedure showed that two components of L-β-Pinene and β-Pinene with MF values of 920 and 906 respectively may exist in this peak cluster. First, to avoid the effect of background and noise in measured data, it is necessary to remove them. The background correction in this work was performed using the method of Liang et al. [17-18]. The selected peak cluster after baseline correction is shown in Figure 4.

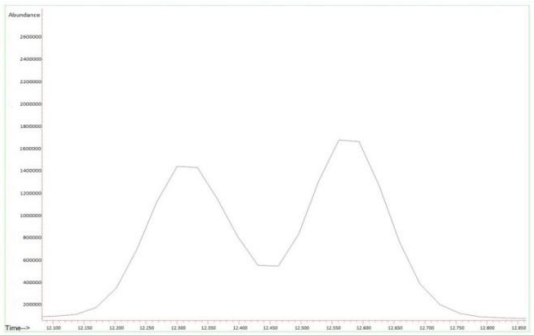

Figure 3: Total ion chromatogram (TIC) of the selected peak cluster

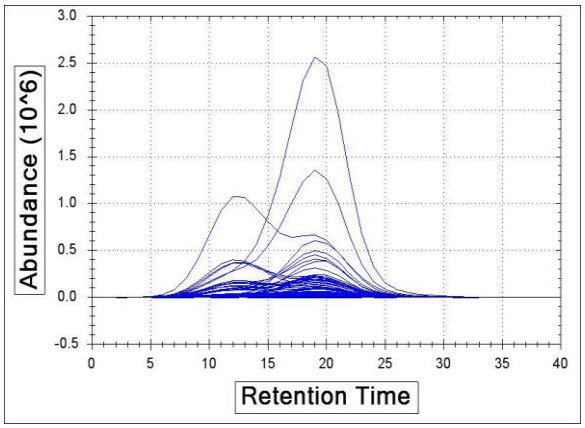

Figure 4: The selected peak cluster after baseline correction

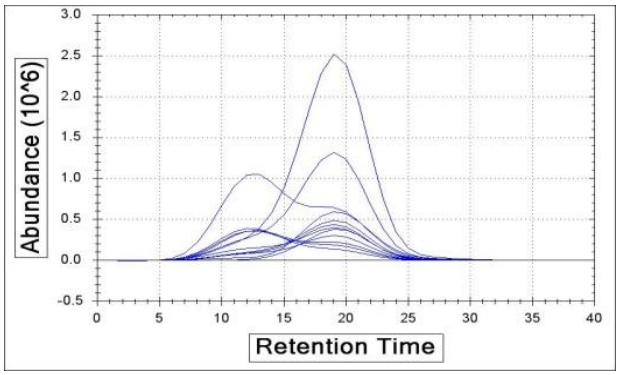

Figure 5: The selected peak cluster after smoothing

Savitzky-Golay filter [19] was used for noise correction, the selected peak cluster after smoothing is demonstrated in Figure 5. These initial steps are necessary for obtaining reliable results in the resolution procedure. Then, the chemical rank determination was done for all peak clusters using methods of subspace comparison and morphological score[16]. Chemical rank determination methods give only an estimation of

the real number of components in the data matrix under study. Therefore, in the chemical rank determination step, the morphological score method which discriminate signal from the noise is used. The results of chemical rank determination for the selected peak cluster are presented in Figure 6 which shows the morphological score against the number of components and illustrates the presence of three components in the peak cluster.

To confirm chemical rank which was determined by morphological score, subspace comparison was used which was shown in Fig.7 and confirmed the presence of three components in the selected peak cluster. After that the purity of two-way data can be determined by fixed size moving window-evolving factor analysis (FSMW-EFA) which is shown in figure 8.

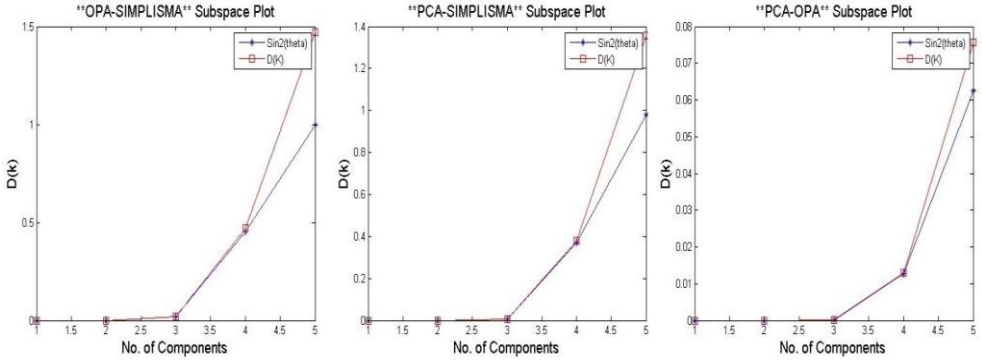

Figure 7: Subspace comparison plots for the selected peak cluster

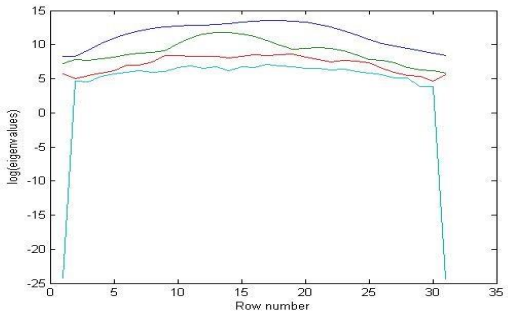

Figure 8: FSMW-EFA plot for peak cluster

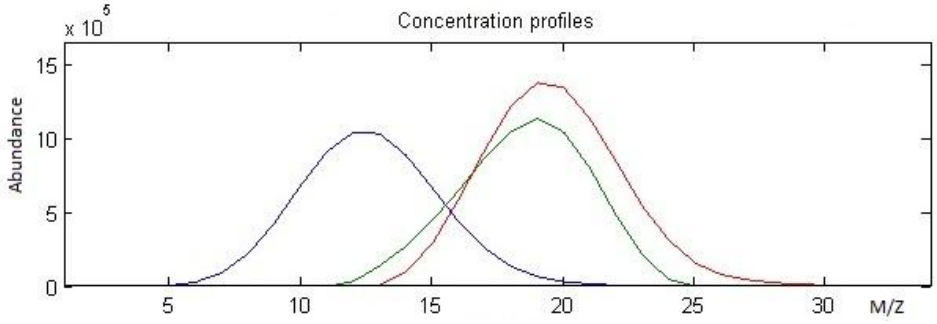

Figure 9: Resolved chromatogram profile of the selected peak cluster

Finally, the peak clusters were resolved using MCR-ALS method. This method is performed with initial estimates of chromatographic profile obtained by EFA method. Constraints of non-negativity and unimodality are applied in ALS algorithm. Pure chromatographic profile and mass spectra for the selected peak cluster obtained using these techniques are shown in Figs. 9 and 10, respectively.

All of these tests showed that there are three components in this peak cluster. After

chemometric analysis more information has been obtained from selected peak cluster and one other component except L-β-Pinene and β-Pinene was identified for selected peak cluster which was β-Myrcene with MF of 901. After extracting each pure spectrum and the resolved chromatographic profiles for each component, the components can be identified by similarity searches using the NIST mass database and can be verified with their RIs. These steps were done for all of peak clusters. Components of green cardamom and bleached cardamom essential oil by GC–MS and MCR method are presented in Tables 1and 2.

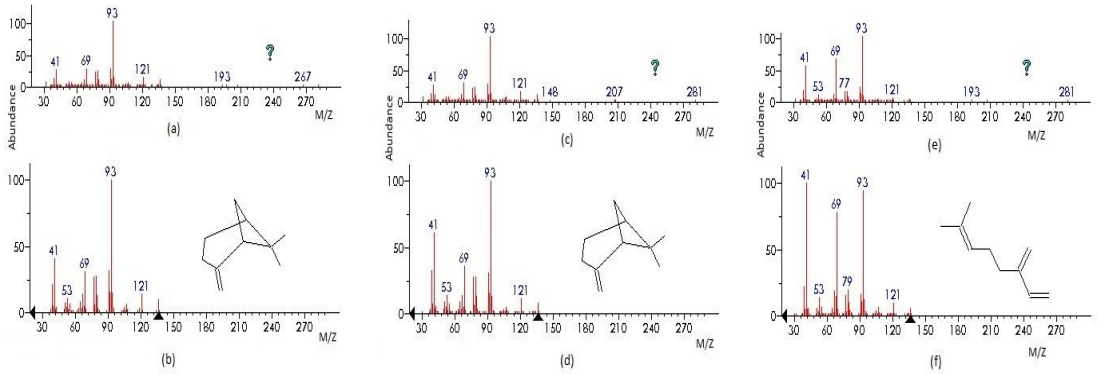

Figure 10: Resolved and standard mass spectra for the selected peak cluster (a) standard and (b) resolved mass spectra of L-β-pinene, (c) standard and (d) resolved mass spectra of β-pinene and (e) standard and (f) resolved mass spectra of β-Myrcene

Table 1: The volatile chemical components of green cardamom essential oil

No.	Compound	% (GC-MS)	% (MCRC)	Retention time	Retention index
1	α-Thujene	-	0.061	91.57	891
2	1R-α-Pinene	0.306	0.294	10.149	905
3	β-Phellandrene	1.063	0.943	11.419	931
4	L-β-pinene	0.323	0.021	12.103	945
5	β-Pinene	0.323	0.072	12.396	951
6	β-Myrcene	-	0.092	12.836	960
7	Sabinene	-	1.993	13.178	967
8	2,3-Dehydro-1,8-cineole	0.092	0.109	13.764	979
9	Octanal	0.174	0.180	14.84	1001
10	Ocimene	-	0.042	15.031	1004.7
11	α-Terpinen	0.215	0.168	15.349	1011
12	1,8- Cineole	54.655	47.175	16.416	1032
13	γ- Terpinen	0.386	0.377	16.873	1041
14	1-Octanol	0.191	0.217	17.482	1053
15	Terpineol , cis-β-	0.782	1.08	17.99	1063
16	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1α,2β,5α)-	0.237	0.151	18.135	1066
17	cis-Linalool Oxide	-	0.096	18.6	1075
18	Terpinolen	-	0.292	19.108	1085
19	Linalool	7.194	6.283	19.819	1099
20	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, cis-	0.075	0.297	20.264	1108
21	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans-	0.161	0.194	20.659	1116
22	Isopinocarveol	-	0.060	21.3	1129

23	Berbenol	-	0.062	21.743	1138
24	L-α-terpineol	0.435	0.388	22.039	1144
25	p-menth-1-en-8-ol	-	0.037	22.335	1150
26	Camphor	-	0.014	22.68	1157
27	L-4-terpineol	2.801	2.477	23.074	1165
28	Terpineol	5.633	4.940	23.42	1172
29	Carvone	0.092	0.222	23.834	1180
30	cis-Piperitol	-	0.080	24.258	1189
31	cis-Carveol	-	0.157	24.94	1203
32	Linalool, formate	-	0.065	25.36	1212
33	Neral	0.462	0.459	26.431	1235
34	Bergamiol	-	0.284	26.757	1242
35	Nerol	-	0.387	27.083	1249
36	Lemonol	1.827	1.807	27.55	1259
37	α-Citral	0.654	0.640	27.876	1266
38	Solanone	0.1	0.127	28.104	1271
39	2-Methyl-oct-2-enedial	-	0.180	28.528	1280
40	α-Terpinyl acetate	16.399	14.331	31.665	1350
41	Geraniol acetate	0.192	0.218	32.106	1360
42	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-	0.175	0.282	32.635	1372
43	Caryophyllene	0.102	0.128	34.576	1417
44	α-Terpinyl propionate	-	0.072	35.115	1430
45	Epoxy-α-terpenyl acetate	-	0.109	35.246	1433
46	2',6'-Dihydroxyacetophenone, bis(trimethylsilyl) ether	0.156	0.327	36.194	1456
47	τ-Muurolene	-	0.093	37.107	1478
48	τ-Selinene	-	0.065	39.631	1541
49	Nerolidol	0.117	0.140	40.182	1555
50	Spathulenol	-	0.096	40.535	1564
51	Caryophyllene oxide	-	0.074	41.361	1586
52	β-Hydroxydodecanoic acid	-	0.126	46.542	1724
53	2,5,5,8a-Tetramethyl-4-methylene-6,7,8,8a-tetrahydro-4H,5H-chromen-4a-yl hydroperoxide	-	0.107	48.21	1771
54	2(1H)-Naphthalenone, 4a,5,6,7,8,8a-hexahydro-6-[1-(hydroxymethyl)ethenyl]-4,8a-dimethyl-, [4ar-(4aα,6α,8aβ)]-	0.104	0.160	52.214	1888
55	Hexadecanoic acid	0.51	0.661	54.837	1968

Table 2: The volatile chemical components of bleached cardamom essential oil

No.	Compound	% (GC-MS)	% (MCRC)	Retention time	Retention index
1	α-Thujene	0.112	0.109	10.784	918
2	1R-α-Pinene	0.432	0.415	11.712	937
3	β-Phellandrene	1.043	0.909	12.299	949
4	β-Pinene	0.224	0.089	12.787	959
5	β-Myrcene	-	0.170	13.129	966

6	L-β-pinene	-	0.019	13.373	971
7	2,3-Dehydro-1,8-cineole	0.335	0.345	14.302	990
8	Octanal	0.105	0.120	14.741	999
9	3-Carene	-	0.061	15.298	1010
10	α-Terpinen	0.281	0.073	16.009	1024
11	m-Cymene	-	0.134	16.314	1030
12	D-Limonene	1.002	0.481	16.67	1037
13	1,8-Cineole	40.666	28.195	17.076	1045
14	γ-Terpinen	0.496	0.460	17.635	1056
15	1-Octanol	0.18	0.195	17.94	1062
16	Terpineol, cis-β-	0.181	0.181	18.295	1069
17	cis-Linalool Oxide	-	0.179	18.702	1077
18	Terpinolen	0.297	0.373	19.108	1085
19	Linalool	7.668	6.389	19.616	1095
20	cis-p-Menth-2,8-dienol	-	0.072	20.018	1103
21	trans-p-Mentha-2,8-dienol	-	0.010	20.636	1110
22	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans-	0.448	0.388	20.757	1118
23	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, cis-	0.101	0.179	21.053	1124
24	cis-p-Mentha-2,8-dien-1-ol	0.065	0.144	21.349	1130
25	Isopinocarveol	-	0.062	21.941	1142
26	1,5,7-Octatrien-3-ol, 2,6-dimethyl-	-	0.097	22.286	1149
27	Octanoic Acid	0.052	0.084	22.532	1154
28	α-Terpineol	6.794	5.562	22.779	1159
29	p-menth-1-en-8-ol	-	0.115	23.025	1164
30	Isoborneol	-	0.044	23.666	1177
31	L-4-terpineol	3.245	0.746	23.962	1183
32	4-Terpineol	-	0.853	24.208	1188
33	p-Cymen-8-ol	0.124	0.159	24.455	1193
34	Cyclohexanol, 2-methylene-5-(1-methylethenyl)-	0.077	0.138	24.701	1198
35	Carvone	0.052	0.153	24.94	1203
36	cis-Carveol	-	0.112	25.313	1211
37	cis-Piperitol	0.077	0.108	25.685	1219
38	trans-Carveol	0.198	0.215	25.918	1224
39	Nerol	6.313	1.473	26.151	1229
40	Unkown	-	0.077	16.431	1235
41	β-Citral	0.306	0.184	26.664	1240
42	Berbenol	-	0.069	26.85	1244
43	Linalool acetate	-	1.383	27.223	1252
44	5-Hepten-1-ol, 2-ethenyl-6-methyl-	-	0.719	27.736	1263
45	α-Citral	0.412	0.401	27.969	1268
46	Cinnamal	0.048	0.084	28.209	1271
47	Cyclohexanone, 2-(2-butynyl)-	0.041	0.28	28.388	1277
48	Solanone	0.157	0.191	28.668	1283
49	trans-3-Caren-2-ol	0.203	0.210	29.367	1298
50	α-Terpinyl acetate	25.521	21.043	29.945	1311
51	Eugenol	0.092	0.135	30.43	1322
52	Geraniol acetate	0.199	0.201	30.739	1329
53	Caryophyllene	0.094	0.135	33.953	1402

54	Terpinyl iso-butyrate	0.066	0.098	34.783	1422
55	Benzoic acid, 3-methyl-2-trimethylsilyloxy-, trimethylsilyl ester	0.101	0.185	37.024	1476
56	β-Cubebene	0.055	0.092	37.439	1486
57	τ-Selinene	-	0.081	38.61	1515
58	Geranyl isovalerate	-	0.075	39.081	1527
59	Nerolidol	0.264	0.225	39.356	1534
60	(S)-(+)-Carvone acetate	0.145	0.032	39.631	1541
61	4-(2,2,6-Trimethyl-bicyclo[4.1.0]hept-1-yl)-butan-2-one	-	0.025	39.906	1548
62	Spathulenol	-	0.083	41.439	1587
63	Caryophyllene epoxide	0.067	0.044	41.911	1599
64	1,2-Longidione	-	0.022	43.932	1653
65	2-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl)but-2-en-1-ol	0.099	0.011	45.279	1689
66	Longifolenaldehyde	0.043	0.149	50.998	1852
67	Diazinone	0.111	0.165	51.572	1869
68	Epimanool	-	0.328	53.957	1941
69	Estradiol, 3-deoxy-	-	0.028	55	1973
70	n-Hexadecanoic acid	0.376	0.150	55.521	1989

Quantitative analysis of green and bleached cardamom essential oil

Peak area integration usually is used to perform qualitative analysis of GC-MS data. After resolving the GC–MS two-dimensional data into pure chromatogram and mass spectrum for each component, the peak area integration at every m/z point for each component can be easily calculated. Its sum, which is called the overall volume integration (OVI) [20, 21], is directly proportional to the concentration of the components. However, the results obtained in the present work are not absolute quantitative concentrations (no standards for all compounds are available) but the percentages obtained after internal normalization of all resolved peak areas [22].

Conclusion

In this work, MAHD technique was applied to extract the volatile components of green and bleached cardamom. Then, the components were characterized by GC–MS. To resolve the peak clusters, the chemometrics method of MCR-ALS was successfully applied. A total of 55 and 70 components with concentrations higher than 0.01 % were resolved using the GC–MS combined with the chemometric resolution techniques for green and bleached cardamom, respectively. However, only 31 and 46 of these components were identified using ChemStation searches in MS database without the use of chemometric resolution techniques. Also, the results show that the hyphenated instruments combined with the chemometric resolution techniques provide a reliable method for the quick and accurate analyses of real samples.

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