Characterization of odor active compounds of fresh and dried turmeric by gas chromatography-mass spectrometry, gas chromatography olfactometry and sensory evaluation

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Abstract

The volatile compounds of fresh and dried turmeric (*Curcuma longa* L.) rhizomes were analyzed by gas chromatography-mass spectrometry (GC-MS), gas chromatography-olfactometry (GC-O) and descriptive sensory analysis (DA). It was found that the extract of fresh rhizome is composed of 31 volatile compounds, the majors being guaiacol, alpha-turmerone and vinylguaiacol, whereas, the extract of dried rhizome is composed of 20 volatile compounds, the majors being alpha-turmerone, vinylguaiacol and guaiacol. By GC-O analyses, we identified eleven and six odor active compounds in fresh and dried rhizome extracts, respectively. Bisabolene and vinylguaiacol had high detection frequency and were selected as specific markers related to sensory attributes. Descriptive analysis revealed that the odor of turmeric was described by 3 sensory terms as turmeric, green and heat.

Keywords: turmeric, aroma, GC-MS, GC-O, descriptive sensory analysis

1. Introduction

Turmeric (*Curcuma longa* L.) is a tropical herb of the Zingiberaceae family. The rhizome of this plant is used worldwide as a dye for industrial coloring and also as a preservative for food. Besides some essential dietary components such as carbohydrates, protein and fiber, turmeric contains also other phytonutrients that play an essential role in human health promotion (Duan *et al.,* 2010; Sandur *et al.,* 2007). The curcuminoid pigments and volatile oils, which are the major secondary metabolites of turmeric, have shown to be responsible for several pharmacological activities. These metabolites are in turmeric powder, turmeric extracts and turmeric oleoresin (Sikkhamondhol *et al*., 2009). Volatile compounds play an important role for the unique taste and smell of turmeric and therefore researches were done on the turmeric volatile compositions. Most of them have focused on the identification

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of major compounds released by this spice using gas chromatography coupled to mass spectrometry (GC-MS). The volatile compounds identified in turmeric were alpha-turmerone, aromatic-turmerone, beta-turmerone, alpha-santalene and aromatic curcumene (Gounder *et al*., 2012; Singh *et al*., 2010). However, the chemical compositions of turmeric volatiles are different considerably among the research groups. Braga *et al*. (2003) found 17 volatiles in turmeric rhizome grown in Brazil with the highest contents of aromatic-turmerone, *cis*-gamma-atlantone, and *trans*-gamma-atlantone. On contrary, Usman *et al.* (2009) reported that the major constituents of turmeric rhizome grown in North Central Nigeria were beta-bisabolene, trans-ocimene, mycene, 1,8-cineole, alpha-thujene and thymol. Recently, Tsai *et al*. (2011) detected different major components in turmeric obtained from Sichuan Province, China such as aromatic-turmerone, humulene oxide and beta-selinene. Despite the large number of volatile compounds found in turmeric, only few of them contribute actively to turmeric flavor. Quantitatively, the major volatile compounds may or may not be important contributors to the aroma, while minor volatiles may have high odor activity (Mahattanatawee *et al*., 2005). GC-MS is an effective way to identify and quantify flavor substance, but it cannot identify odor-active compounds, which are also called odorants. Gas chromatography-olfactometry (GC-O) is a valuable method for examining the pattern of odorants in terms of their odor descriptors and activity. Although many papers revealed the volatile compounds of turmeric, GC-O studies of turmeric samples are scarce. Another valuable tool for specifying the characteristics of complex aromas is descriptive analysis (DA). DA techniques employ a panel to specify the intensities of specific attributes. In this process, it has been demonstrated that panelists are capable of describing their perceptions and are able to reach an agreement through discussion as to align the concepts of each participant and to obtain set of words uses as representation of the sensations that they have experienced (Lawless, 1999). The aims of the present study were to analyze the volatile compounds of turmeric extracts and their odor-active compounds and to describe the odor attributes of turmeric. Through the data obtained from descriptive sensory analysis, GC-O and GC-MS, the desirable odor active compounds with appropriate descriptors were recommended as turmeric contributors, aiming to determine the most important marker compounds, which could be used to follow the change of odor character of turmeric during processing, final product formulation and packaging of turmeric-containing products.

2. Materials and Methods

2.1 Plant materials

Fresh turmeric rhizomes used in the present study were purchased from a local market in Chiang Mai province, Thailand. The rhizomes were manually cleaned with water to remove the adhering soil and extraneous matters. These clean and fresh rhizomes were thinly grated, and divided into two parts. The first part was subjected to a blender to obtain fine paste. It was referred as 'fresh' rhizome sample. The second part was dried at 50°C in a hot air oven (Binder, USA) for 24 h. Then, the dried rhizomes were grounded to fine powder having a moisture content of 14.94% on dry weight basis. This sample was referred as 'dried' rhizome sample.

2.2 Preparation of extracts

Extracts were prepared using microwave assisted extraction (MAE) as described by Laokuldilok *et al*. (2012). Briefly, each sample (25 g) was mixed with 500 ml of 95% ethanol (Bangkok alcohol, Thailand) and placed in the center of microwave oven (Toshiba, Model ER-300C(S), Power Max 900 W, frequency 2.45 x 109 Hz). The suspension was exposed to 900 watts of microwave power for 1 min. After extraction, the solvent was evaporated under vacuum using a rotary evaporator (Büchi Rotavapor R-200, Switzerland) at 40°C. The fresh and dried turmeric extracts were kept in air-tight amber bottles after flushing with nitrogen gas for 30 s and stored at 4°C for further analysis. The extraction yields of dried and fresh turmeric were 18.52% and 15.74% on dry weight basis, respectively.

2.3 Characterization of volatile compounds in turmeric extracts

Prior to analysis, each turmeric extract was dissolved with absolute methanol (RCI Labscan Limited, Thailand) to obtain the concentration of 30 mg/L then filtered through a 0.45 µm filter (MS® Syringe filter, U.S.A.). The two extracts were directly injected in the injection port of an Agilent gas chromatograph series 6890 equipped with quadruple mass spectrometer (MS, 5973, Agilent Technologies, USA) fitted with an HP-5MS column (30 m × 0.25 mm × 0.25 µm film thickness, Agilent Technologies, Inc., USA). Helium gas was used as a carrier gas at a flow rate of 1 ml/min. Samples in volume of 1 µl were injected with a split ratio of 1.5:1. The temperature program was started by warming the column at 50°C for 5 min, heated to 200°C at a rate of 10°C/min and then increased to 250°C at a rate 5°C/min and held at 250°C for 10 min. Volatile compounds were fragmented using electron-impact ionization (70 eV), with a source temperature of 230°C; the mass scan range was 30–500 amu. Volatile compounds were identified based on comparison of mass spectra with those of spectral libraries NIST 05

and Wiley 7N Registry of GC Mass Spectral Data (John Wiley, New York, USA). Linear retention indices (LRI) were calculated for each compound against *n*-alkane standards (C_8–C_{20}) and compared with that of the published values in literature (NIST Chemistry web book). The extracts were also run by gas chromatograph-flame ionization detector (GC-FID, Shimadzu 2010, Japan) with the same condition as GC-MS and were calculated for the quantities of individual components using the effective carbon number concept (Faiola *et al*., 2012).

2.4 Characterization of odor-active compounds by gas chromatography-olfactometry analysis (GC-O)

To characterize odor-active compounds, a gas chromatograph (GC-2010, 05853, Shimadzu, Japan) coupled with an olfactometer sniffing port (O275, 1017, GL Sciences Inc., Japan) was used. The GC operating conditions were the same as mentioned in 2.3. One µl of prepared sample was injected into an injection port, connected to a DB-5 column (30 m x 0.53 mm, i.d., 1.50 µm film thickness). Detection frequency method (DFA) using three experienced female panelists were applied to obtain the odor profile of turmeric. Each of the three panelists participated in perceiving the aroma compounds separated from turmeric at the sniffing port. The panelists were asked to give verbal description of perceived odors that were recorded. The number of times an odor compound was sensed at a given retention time by the panelists was counted (Plutowska and Wardencki, 2008).

2.5 Sensory evaluation

Fresh and dried turmeric rhizomes were evaluated for their intensity of dominant odors using descriptive sensory analysis (DA) which was conducted by adapting the method of Stone (1992). Ten trained panelists (7 females and 3 males) were selected from graduated students in the Division of Product Development Technology, Faculty of Agro-Industry, Chiang Mai University, Thailand. The panelists received 8 h of training, consisting of four 2-h sessions conducted over 2 weeks in order to determine the consensus list of odors, basic tastes and the references for each descriptor. Sensory sessions took place in a sensory laboratory, which complied with standards for test room. In the first session, panelists were specifically asked to describe the odor perceived and to memorize the perception; in the second and third, different aroma references were presented and discussed by panelists. From these discussions, the aroma terms and their references were selected for further descriptive analysis. The prepared reference of each attribute was then rated on an unstructured 152 mm line scales anchored by mark 12.7 mm from either end (Stone, 1992). The list of descriptors, definitions and references are shown in Table 1. In the fourth session, the panelists were trained to assess warm-up

sample using the same sensory sheets to be used in the main sensory evaluation. After the individual assessment for the sample, open discussion was held to assess the evaluation results and reach agreement for perceived aroma intensity of the provided sample.

Table1 Attributes, standard references, and ratings used in descriptive sensory analysis of fresh and dry turmeric

Attribute	Definition	Reference	Rating (mm.)
Turmeric	Aromatic associated with turmeric root	0.10% (v/v) of turmeric oil in propylene glycol (AromaMore Ltd., Thailand)	79
Green	Aromatic associated with peel of freshly pomelo	0.20% (%v/v) of bisabolene standard in propylene glycol (CAS: 495-62-5) (Alfa Aesar, A Johnson mothey Co., Ltd, United Kingdom)	83
Heat	Chemical burning, sensation in the nasal	0.20% (%v/v) vinylguaiacol standard in propylene glycol (CAS: 7786-61-0) (SAFC, United Kingdom)	74

Sensory evaluation was performed with randomly order and coded with 3-digit random numbers. Ten panelists participated to the main evaluation, which was carried out in individual testing booths equipped with a computerized program SUsense (Silpakorn University, Nakhon Pathom, Thailand) for experiment setting and data collection. Before sample evaluation, the panelists spent a few minutes familiarizing themselves with the anchored references and a warm-up sample (Siriruangampai brand, Doi Saket, Chiang Mai, Thailand) was presented for panelist calibration. The intensity of each attribute was then rated on an unstructured 152 mm line scale anchored at 12.7 mm from each end (Stone, 1992) labeled with ”Slight” on the left and ”Strong” on the right. Data were quantified as distance from the origin in the millimeters and the average of all the panelists was calculated for each sample. A container including coffee beans was provided to each panelist, who was asked to sniff to minimize sensory adaptation.

2.6 Statistical analysis

Data from the descriptive sensory analysis was evaluated by analysis of variance (ANOVA) using SPSS v16.0 (SPSS Inc., Chicago, IL, USA). ANOVA with Duncan's multiple range tests were performed to determine the difference among individual sample for each sensory attribute.

3. Results and Discussion

3.1 Characterization of volatile compounds in turmeric extracts

The chromatograms of extracts obtained from fresh and dried turmeric are shown in Figure1. The characteristic peaks were identified by comparing the mass spectra with literature (NIST Chemistry web book) and also shown in Table 2. Totally, there were 31 compounds detected in the extract of fresh rhizome including 1 ether, 4 alcohols, 11 alkenes, 4 ketones, 6 esters, 1 aldehyde and 4 fatty acids. The major components were guaiacol, alpha-turmerone and vinylguaiacol. These findings are in good agreement with previous reports in which alpha-turmerone was identified as major components of turmeric (Gounder and Lingamulla, 2012; Singh *et al*., 2011). Twenty components were identified in the extract of dried turmeric rhizome including 5 alcohols, 6 alkenes, 4 ketones, 2 esters, 1 aldehyde and 2 fatty acids. The major compounds were alpha-turmerone, vinylguaiacol and guaiacol. It is interesting to note that the concentration of alpha-turmerone was lower in the fresh rhizome, when compared to that of dried rhizome. In addition, the results showed that aromatic-turmerone appeared as a minor compound in dried rhizome. This may be due to rearrangement and oxidation of less stable alpha-turmerone to the most stable aromatic-turmerone (Gounder and Lingamulla, 2012).

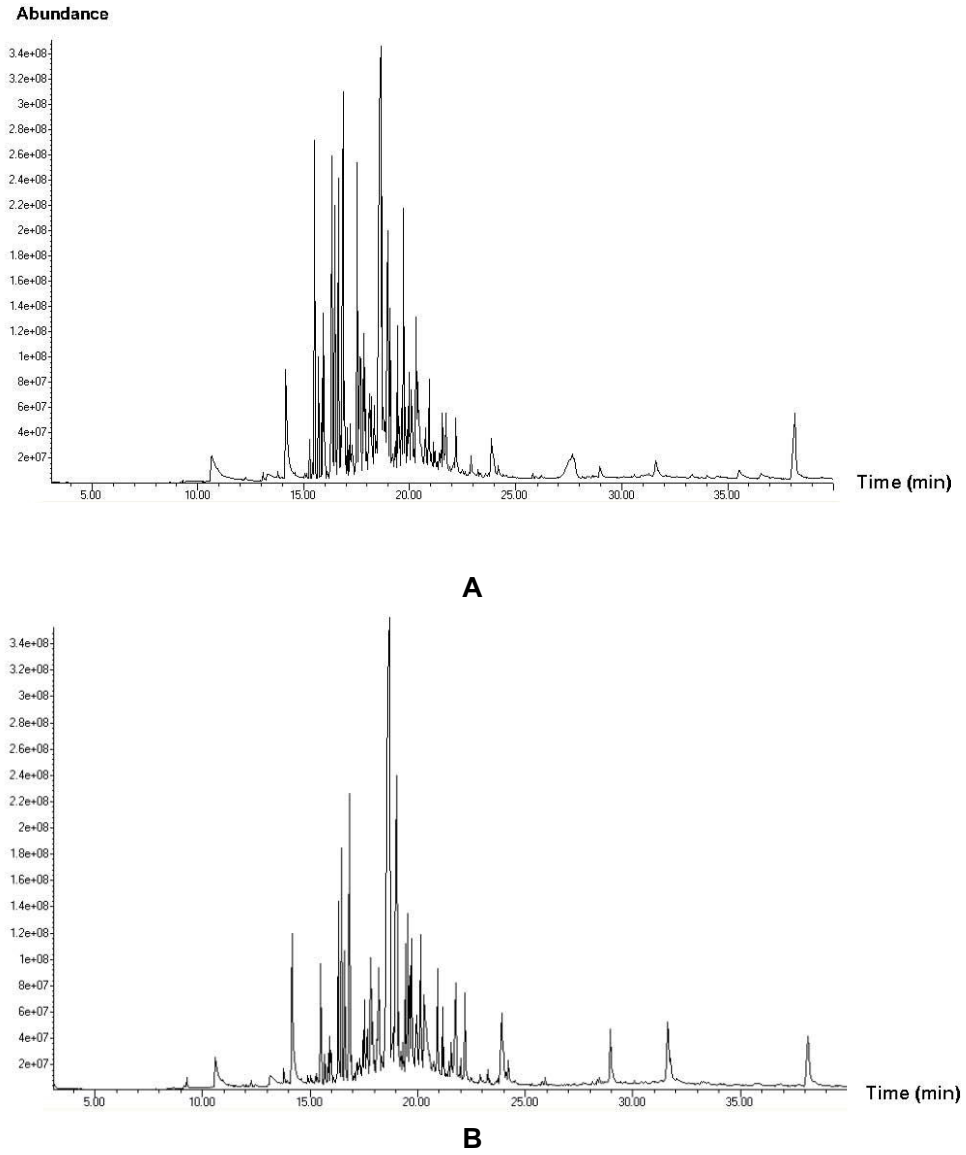

Figure 1 Chromatograms of extracts obtained (B) from (A) fresh turmeric and (B) dried turmeric rhizome.

Table 2 Volatile compounds and their contents in fresh and dried turmeric extracts

LRI	Compounds	Fresh	Dried
1028	Eucalyptol	0.34	-
1090	Guaiacol	54.51	14.77
1188	alpha-Terpineol	0.33	-
1312	Vinylguaiacol	22.20	20.85
1380	(*Z*)-beta-Elemene	-	0.08
1383	2-epi-alpha-Cedrene	0.18	-
1428	alpha-Santalene	0.98	-
1433	(*E*)-alpha-Bergamotene	0.45	4.94
1451	Caryophyllene	0.35	2.31
1480	beta-Farnesene	0.42	-
1483	*ar*-Curcumene	2.50	-
1488	gamma-Curcumene	0.31	-
1511	(*Z*)-alpha-Bisabolene	-	0.50
1520	Sesquiphellandrene	0.18	-
1525	beta-Bisabolene	2.31	1.75
1531	(*Z*)-gamma-bisabolene	0.22	-
1538	gamma-Cadinene	8.85	-
1564	Nerolidol	-	0.47
1583	(*Z*)-alpha-Santalol	-	0.86
1596	beta-Elemenone	-	3.27
1618	Zingiberenol	-	2.16
1632	aromatic-Turmerone	-	1.25
1654	alpha-Tumerone	25.16	26.82
1648	Curlone	8.42	4.70
1735	(*E*)-alpha-Atlantone	2.20	-
1742	4-(4-hydroxy-3-methoxyphenyl)-3-Buten-2-one	11.47	-
1877	Methyl isohexadecanoate	0.43	-
1964	n-Hexadecanoic acid	2.26	1.53
1981	Hexadecanoic acid, ethyl ester	0.34	-
2031	1,2- Hexadecanediol	0.11	-

Note: LRI = Linear retention index, NA = data not available

* calculated according to the method of Faiola *et al*. (2012)

Table 2 Volatile compounds and their contents in fresh and dry turmeric extracts (cont.)

LRI	Compounds	Fresh	Dry
2112	Methyl-8,11- octadecadienoate	0.12	-
2297	(*Z*)-9,17-Octadecadienal	0.45	0.65
2134	Linoleic acid	1.40	0.46
2143	(*Z*,*Z*,*Z*)-9,12,15-Octadecatrienoic acid	0.43	-
2152	Ethyl-9,12-octadecadienoate	0.14	-
2179	Stearic acid	0.35	-
2185	Ethyl-2-hydroxy-1-(hydroxymethyl) hexadecanoate	1.30	0.36
2191	Ethyl-2-hydroxy-1-(hydroxymethyl), (*Z*,*Z*)-9,12	1.73	0.33

Note: LRI = Linear retention index, NA = data not available

* calculated according to the method of Faiola *et al*. (2012)

The chemical composition of the volatiles was found to be different from other geographical locations. Usman *et al.* (2009) reported the presence of 22 compounds in the rhizome of North Central Nigeria, among which the majors were beta-bisabolene (13.9%), trans-ocimene (9.8%) and mycene (7.6%). Tsai *et al*. (2011) reported aromatic-turmerone (49.04%), humulene oxide (16.59%) and beta-selinene (10.18%) as major constituents in volatile compounds from dried rhizome of *C. longa* imported from Sichuan Province, China. Volatile compounds of turmeric from Brazil were isolated by hydrodistillation, low pressure solvent extraction and supercritical extraction (Braga *et al*., 2003). The major compounds were aromatic turmerone, (*Z*)-gamma-atlantone, and (*E*)-gamma-atlantone. As observed in the above studies, the volatile components obtained from the rhizomes grown at different geographic regions and climatic conditions, showed considerable difference in their pattern of chemical composition (Usman *et al*., 2009). The chemical composition of the turmeric rhizome depends on the genotype, field conditions, and postharvest processing of the rhizomes (Cousins *et al*., 2007).

3.2 Characterization of odor-active compounds by GC-O

GC-O evaluation demonstrated the presence of 11 odor active peaks in the volatile extract obtained from fresh turmeric for which the odor descriptors are listed in Table 3. Five odor active compounds were characterized with high detection frequency. These compounds have already been reported as aroma contributors: eucalyptol (eucalyptus), guaiacol (smoke, sweet, medicine), vinylguaiacol (apple, spicy, peanut, wine-like or clove and curry), trans-alpha-bergamotene (wood, warm, tea) and bisabolene (warm, spicy, balsamic) (Brechbill, 2006).

Table 3 also shows the presence of 6 odor active compounds found in dry turmeric rhizome. Two odor active compounds *i.e.* vinylguaiacol and trans-alpha-bergamotene were recognized in dry turmeric rhizome with high detection frequency. These two compounds also were the aroma contributors in fresh rhizome. As seen in Table3, the descriptor of each compound was slightly different among panelists. Delahunty *et al*. (2006) reported that different assessors may describe the same sensation in different ways. A panelist's initial description of aroma compound is based upon the assessor's experience. In addition, compounds are described in terms of previously experienced food or other volatile substances (Mahattanatawee and Rouseff, 2011).

Table 3 Odor-active compounds of fresh and dry turmeric determined by gas chromatography-olfactometry, the compounds' detection frequencies (3 assessors)

LRI	Compounds	Characteristic odor[a]	Fresh turmeric[b]				Dry turmeric			
			Panelist 1	Panelist2	Panelist3	DF[c]	Panelist1	Panelist2	Panelist3	DF[c]
957	Eucalyptol	eucalyptus, herbal, camphor	herbal	mint	herbal	3	-	-	-	-
993	Guaiacol	smoke, sweet, medicine	smoke	burn	medicine	3	burn	-	-	1
1286	Vinylguaiacol	spicy, clove, smoky, woody	spice	heat	spice	3	spice	dried chili powder	pungent	3
1448	(*Z*)-alpha-Santalol	sweet-woody, balsamic odor	woody	-	-	1	woody	-	sweet	2
1462	(*E*)-alpha-Bergamotene	warm	warm	warm	wood	3	warm	wood	wood	3
1466	Caryophyllene	spiciness of black pepper	spice	spice	-	2	-	spice	-	1
1482	Sesquiphellandrene	sweet, fruity, herbaceous, woody	-	-	herbal	1	-	-	-	-
1527	beta-Farnesene	citrus, green	green	-	-	1	-	-	-	-
1585	bata-Bisabolene	citrus, fruity	citrus	citrus peel	citrus	3	-	citrus	citrus peel	2
1708	n-Hexadecanoic acid	slightly waxy fatty	fatty	fatty	-	2	-	-	-	-
1803	(*Z*,*Z*)-9,12-Octadecadienoic acid	faint fatty	fatty	fatty	-	2	-	-	-	-

Note: LRI = Linear retention index, DF = Detection frequency [a] www.thegoodscentcompany.com , [b] odor description generated by each panelist

None of the peaks were representative of the characteristic odor of turmeric. The odor appeared to be an integration of a complex mixture of odor compounds. Some odor active compounds were present at low concentrations that were difficult to detect by MS. Moreover, the results also demonstrated that many volatile compounds had little to no odor activity. The literatures showed that turmerone was mainly responsible for characteristic odor of turmeric with slightly pungent bitter taste (Tsai *et al*., 2011). However, it had no aroma activity in this study; thus it is not listed for odor descriptors.

3.3 Sensory analysis

The fresh and dried turmeric rhizomes were investigated for their characteristic odors. Descriptive sensory analysis was provided the term, definition and intensity of each interest odor. The results from panelists' detection showed that the odors of fresh and dry turmeric were detected with the similar terms *i.e.* turmeric, green and heat. During the second and third training session, the references at different concentrations were prepared for panelists to evaluate and discuss the intensity of each attribute on an unstructured line scale. The consensus on intensity of references was then evaluated and the panelists were continually trained until the intensity scores were stable. The intensities of fresh and dried turmeric have reached agreement. The three attributes seemed to well explain their aroma characteristics of different samples. ANOVA analysis indicated significant differences ($p < 0.05$) among samples in the intensity of the 3 attributes. The mean intensity values of the 3 attributes and the results of Duncan's multiple comparison tests are shown in Table 4.

Table 4 The mean intensity values of the 3 attributes for fresh and dry turmeric in descriptive sensory evaluation[a].

Sample	Attributes		
	Turmeric (mm.)	**Green (mm.)**	**Heat (mm.)**
Fresh turmeric	56.00a	40.63a	42.88a
Dry turmeric	16.00b	7.44b	5.38b

Note: [a] Mean scores for each attribute within a column with different letters are significantly different ($p \leq 0.05$) using Duncan's multiple comparison tests ($n = 20$; 10 panelists with 2 replications).

The results (Table 4) showed that fresh turmeric displayed higher intensity for all attributes. The green attribute described by using the mixer of bisabolene isomers as a reference was rated higher in fresh turmeric. This result is in agreement with the GC-MS analysis; bisabolene and its isomer of fresh turmeric were present in higher amounts than that of dried turmeric, which were 2.53 and 2.25 µg/g extract, respectively. Heat attribute, which used vinylguaiacol as a reference, was also rated higher in fresh turmeric. This result was in agreement with GC analysis which revealed that fresh turmeric displayed higher content of vinylguaiacol (22.20 µg /g extract), when compared to dried turmeric (20.85 µg /g extract).

Although dried food can be preserved for a long time, the drying process has effect on the quality of dehydrated products. The lower amount of volatile compounds in dried turmeric may indicate that the drying process affects on the stability of volatile compound. On the other hand, in the present experiment, the seven new volatile compounds were detected in dried turmeric such as (*Z*)-beta-elemene, (*Z*)-alpha-bisabolene, nerolidol, santalol, beta-elemenone, zingiberenol and aromatic turmerone. The presence of new volatile compounds in dried turmeric could be mainly explained with the chemical and biochemical reactions occurring in turmeric during drying (Ruse *et al*., 2012).

4. Conclusion

Descriptive sensory analysis along with GC analysis was useful to monitor and describe changes in aroma characteristics of fresh and dried turmeric. Moreover, well trained panelists could be used as the great indicator in precisely analyzing the aroma profile in the sample, which cannot be achieved with any other analytical instrument. The two peaks in GC chromatogram, which were recognized as odor active compounds in GC-O analysis and at the same time had peak areas that were correlated with the characteristic odors analyzed by DA, were selected as the reference standards to represent “green” and “heat” characteristic odors in fresh and dried turmeric. The change of aroma character of turmeric during processing, final product formulation and packaging of turmeric-containing products can be followed using the odor active compounds found in this study.

Acknowledgements

The authors gratefully acknowledge the National Science and Technology Development Agency (NSTDA) for providing financial support.

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