

Levels of curcuminoid and essential oil compositions in turmeric (*Curcuma longa* L.) grown in Korea

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Abstract Levels of curcuminoids and essential oil compositions from eighteen turmeric (*Curcuma longa* L.) grown in Jeju were analyzed. 0.5 g of turmeric samples was extracted with 20 mL of methanol for 7 h by shaking and was ultra-sonicated for 30 min. The extracts were analyzed by high-pressure liquid chromatography-diode array detector using Brownlee SPP C18 column (4.6 × 100 mm, 2.7 μm) after filtration. Curcumin (CUR), demethoxycurcumin (DEM), and bisdemethoxycurcumin (BIS) were monitored at the wavelength of 420 nm. Total curcuminoids in turmeric ranged from 53.9 ± 1.0 to 5396.0 ± 101.8 μg/g dry turmeric weight. In decreasing order, curcuminoids found were CUR (4669.7 μg/g) > DEM (565.3 μg/g) > BIS (160.9 μg/g). K145023 variety contains the highest amount of curcuminoids and variety number 10 contains the least amount. Essential oils were extracted with steam distillation method and analyzed with GC-MS. The major components of turmeric oil were α-zingiberene (27.70–36.75 %), aromatic-turmerone (19.54–32.24 %), β-sesquiphellandrene (13.14–18.23 %), α-turmerone (3.72–6.50 %), β-turmerone (2.86–5.60 %), and β-bisabolene (2.50–3.46 %).

Keywords Turmeric · Curcumin · Demethoxycurcumin · Bisdemethoxycurcumin · *Curcuma longa*

Introduction

Turmeric (*Curcuma longa* L.) is a tropical herb of the Zingiberaceae family indigenous to southern Asia. The dried ground rhizome of the perennial herb *Curcuma longa* Linn., called turmeric in English, haldi in Hindi, and ukon in Japanese, has been used in Asian medicine since the second millennium BC (Brouk 1975). It has been used as an important food ingredient in India for thousands of years because of its special aromatic flavor and attractive yellow color (Singh et al. 2010). It is still used world widely in food as a condiment, particularly as an essential ingredient of curry power, in medicine due to its beneficial effects, such as bilious regulating function (Ramprasad and Sirsi 1956, 1957), anti-inflammatory (Arora et al. 1971; Ghatak and Basu 1972; Chang and Fong 1994), antiarthritic (Sambaiah et al. 1982), and increasing of insulin secretion (Wickenberg et al. 2010). Turmeric is also used in drug against cancer dermatitis, AIDS, and high cholesterol level (Kuttan et al. 1985; Ammon and Wahl 1991; Azuine and Bhide 1992).

The distinct yellow color of turmeric comes from the pigment curcumin (CUR), 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, and two curcuminoids, demethoxycurcumin (DEM) and bisdemethoxycurcumin (BIS) (Fig. 1). These compounds are used in the food industry as a natural dye. Many researchers have shown that curcumin and curcuminoids have various biological activities, such as antioxidant, anticancer, anti-arthritis, anti-inflammatory activities (Selvam et al. 1995; Hanif et al. 1997; Sharma et al. 2005). Curcuminoids also have

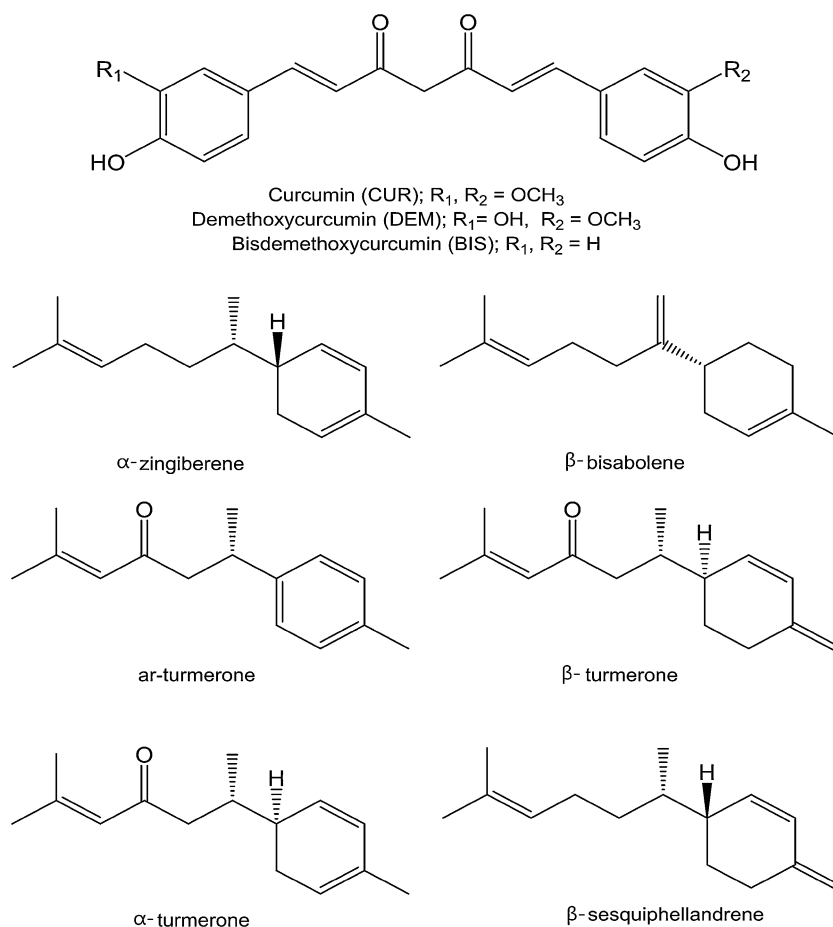
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Fig. 1 The chemical structures of curcuminoids and major constituents of turmeric essential oil



bacteriostatic effect against *Staphylococcus aureus* (Ramprasad and Sirsi 1957; Park et al. 2005), antifungal (Kim et al. 2003), blood glucose level suppressing effect (Nishiyama et al. 2005), and effect against Alzheimer's disease (Park and Kim 2002).

The essential oil of turmeric contains aromatic-turmerone (ar-turmerone), β -turmerone, α -turmerone, α -zingiberene, β -sesquiphellandrene, and β -bisabolene (Sharma et al. 1997; Neela et al. 2002; Manzan et al. 2003; Jayaprakasha et al. 2005; Singh et al. 2010). The essential oil from the rhizomes of turmeric shows biological activities of antibacterial, antifungal, anticancer, insect repellent, and anti snake venom activity. The essential oil extracted from turmeric also possesses anti-inflammatory, antifungal, antihepatotoxic, and antiarthritic activities (Arora et al. 1971; Kiso et al. 1983; Polasa et al. 1992; Behura et al. 2000; Funk et al. 2010; Singh et al. 2010).

Recently, turmeric has been cultivated in the southern part of Korea including Jeju and Jeonnam (Kim et al. 2005, 2013). The level of curcuminoids and the compositions of essential oil are the important criteria of the quality and utility of a variety with respect to its use as a source of the health-promoting agent. The secondary metabolite profile

is affected by climate, the location of the plant cultivated. To select the most adequate variety that has the most abundant secondary metabolites, we analyzed curcuminoids and essential oils from eighteen varieties of turmeric grown in Jeju.

Materials and methods

Materials and chemicals

CUR, DEM, and BIS were purchased from Sigma-Aldrich Chemical Co. (USA). High-pressure liquid chromatography (HPLC) grade methanol and acetonitrile were purchased from Fisher Co. (USA). Stock solutions of curcuminoids (1.0 mg/mL) were prepared in methanol and diluted with methanol to prepare standard solutions of 1.0, 5.0, 10.0, 50.0, 100.0, and 200.0 $\mu\text{g/mL}$ concentrations. Eighteen varieties of turmeric were provided from the National Agrobiodiversity Center and were grown in Jeju Island from mid February to end of December in 2013 and rhizomes were separated after harvest. The roots were washed, sliced 5 mm width, and dried 48 h at 45 °C in

drying oven. After drying, the samples were pulverized with a blend mixer and kept in a refrigerator at $-20\text{ }^{\circ}\text{C}$ until analysis.

Sample preparations for curcuminoids analysis from turmeric

Powders (0.5 g) of the dried turmeric were extracted with 20 mL of methanol for 7 h with a mechanical shaker at room temperature after 30-min ultrasonication. The extracted solutions were filtered with an Acrodisc Syringe Filter with $0.2\text{ }\mu\text{m}$ HT Tuffryn membrane (USA), and the sample was stored at $-20\text{ }^{\circ}\text{C}$ until used for the curcuminoids analysis.

Analysis of curcuminoids

Quantitative analyses of curcuminoids were performed using an Agilent 1100 model HPLC system equipped with a Brownlee SPP C18 column ($100\text{ mm} \times 4.6\text{ mm}$ i.d., $2.7\text{ }\mu\text{m}$ particle) and a diode array detector (DAD). Mobile phase A was water and B was acetonitrile. The isocratic mode was set at A/B ratio of 70/30. The UV absorption spectra of each curcuminoids were monitored with DAD, and the detection wavelength was set at 420 nm for curcuminoid with maximum absorption. The flow rate was 0.8 mL/min, and 10 μL of sample was injected to HPLC for quantitative analysis. Concentrations of curcuminoids were calculated using the regression equation of their concentration and peak area.

Essential oil extraction

Essential oils were extracted with steam distillation method. Powders (50 g) of the dried turmeric were mixed with 500 mL of water and oils were extracted with steam distillation apparatus at $120\text{ }^{\circ}\text{C}$ for 2 h. Extracted oils were separated from water layer and residual water was removed by adding anhydrous sodium sulfate and kept in the refrigerator for overnight. The weight of oil was measured and stored at $-20\text{ }^{\circ}\text{C}$ until analysis. 10 μL of oil was added into *n*-hexane (1 mL) before analyzing with gas chromatography–mass spectrometry detector (GC/MSD).

Essential oil composition analysis

Compounds in essential oil were identified by comparison with the Kovats' gas chromatographic retention index (*KI*) and by the mass spectral fragmentation pattern of each component compared with those of authentic compounds. The identification of the GC components was also conducted with the NIST AMDIS version 2.1 software.

An Agilent model 6890 GC interfaced to an Agilent 5971A mass selective detector (GC/MS) was used for mass spectral identification of the GC components at MS ionization voltage of 70 eV. An Agilent model 6890 GC equipped with a $30\text{ m} \times 0.25\text{ mm}$ i.d. ($df = 0.5\text{ }\mu\text{m}$) DB-5MS bonded-phase fused silica capillary column (Agilent, USA) was used for measurement of KI and routine analysis of essential oils. The helium carrier gas flow rate was 1.0 mL/min at a split ratio of 20:1. The injector and detector temperatures were $250\text{ }^{\circ}\text{C}$ and $280\text{ }^{\circ}\text{C}$, respectively. The oven temperature was programmed from $40\text{ }^{\circ}\text{C}$ (held for 5 min) to $210\text{ }^{\circ}\text{C}$ at $2\text{ }^{\circ}\text{C}/\text{min}$ and then held for 70 min.

Results and discussion

Levels of curcuminoids

To select the maximum absorption wavelengths, UV absorption spectra were monitored by HPLC–DAD. All curcuminoids have maximum absorption at 420 nm, and CUR has the highest absorption followed by DEM and BIS. For the monitoring of curcuminoids in HPLC analysis with DAD, 420 nm of wavelength was selected. The evaluation of total amount of curcuminoids in sample by use of direct absorption measurement at 420–430 nm was used, but this method is not valid if there are other compounds absorbing in the region of 420–430 nm. And the absorption coefficients of curcuminoids are not identical. So, it is not possible to quantify the individual curcuminoids with spectrophotometric method (Jayaprakasha et al. 2005). The HPLC could be a solution. Figure 2 shows the HPLC chromatogram of curcuminoid's standard solution (A, 50 $\mu\text{g}/\text{mL}$) and sample extract obtained from K145023 (B) variety. The retention times of each compound from the analytical condition were 12.21, 13.59, and 15.08 min for BIS, DEM, and CUR, respectively. The calibration curves for three curcuminoids showed good linearity from 1.0 to 200 $\mu\text{g}/\text{mL}$ concentration with the correlation constant over 0.999.

Curcuminoids in turmeric could be extracted with high yield using ultrasonication in methanol (Lee et al. 2012), so, we extracted dried turmeric with methanol by shaking for 7 h after 1 h ultrasonication. The levels of curcuminoids in various turmeric varieties grown in Korea are shown in Table 1. The total curcuminoids levels were ranged from N.D. to $5396.0 \pm 101.8\text{ mg}/\text{kg}$ and K145023 variety contains the highest level of curcuminoids. Eleven varieties had more than 3575.5 mg/kg but the other seven varieties had less than 468.1 mg/kg of total curcuminoids. The decreasing order of total curcuminoids was K145023 ($5396.0 \pm 101.8\text{ mg}/\text{kg}$) > K134857 > K134897 > Var

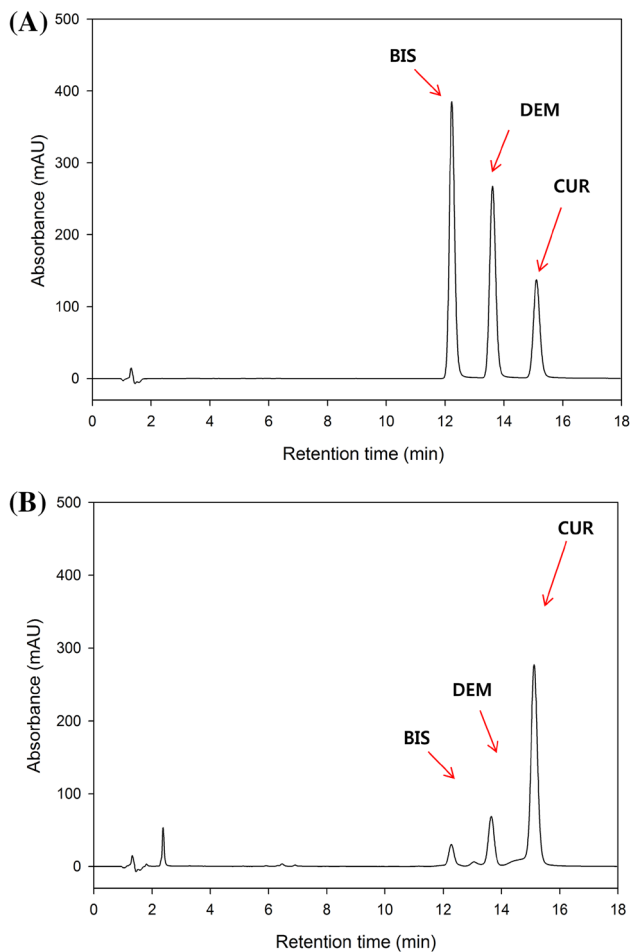


Fig. 2 Typical HPLC chromatogram of a standard solution (A) and a methanol extract obtained from turmeric K145023 variety (B)

20 > K134786 > Var 2 > K134957 > Var 4 > K135723 > K134741 > Var 12 > K134956 > Var 14 > Var 7 > Var 17 > Var 10 > Var 13, KNP050602 (N.D.). Among curcuminoids, CUR was found in the greatest amounts with detection ranges from N.D. (Var 13, KNP050602) to 4669.7 ± 89.3 mg/kg (K145023) followed by DEM from N.D. to 565.5 ± 9.6 mg/kg, and BIS from N.D. to 161.0 ± 2.9 mg/kg, respectively (Fig. 3). These results on the curcuminoids level in turmeric were similar to the previous reports (Kim et al. 2005; Green et al. 2008; Lee et al. 2012).

Essential oil Composition

The essential oil contents in three dried rhizomes of *C. longa* were ranged from N.D. to 13.9 mg/g dry weight (Table 1). The essential oil content in the variety K134857 was the highest as 13.9 mg/g dry weight followed by K134897 (13.5 mg/g), K145023 (13.0 mg/g), Var 20 (12.6 mg/g), K134957 (9.5 mg/g), Var 2 (7.9 mg/g),

K135741 (7.8 mg/g), K134786 (7.3 mg/g), Var 12 (5.0 mg/g), K134723 (4.6 mg/g), and Var 4 (1.1 mg/g). But other seven varieties contained too small amount of oil to extract. It is interesting that the essential oil contents were proportional to the level of curcuminoids in turmeric. For example, total curcuminoids in K145023 is 5396.0 mg/kg, and the essential oil content is 13.0 mg/g, while Var 4 has 3717.8 mg/kg curcuminoids and 1.1 mg/g oil.

The essential oil content in dried rhizome of Indian *C. longa* was 9.85 mg/g dry weight (Tsai et al. 2011) and Raina et al. (2002) reported the essential oil of *C. longa* rhizomes to be 2.2 % (equivalent to 22 mg/g) dry weight. It is thought that the cultivar and breeding location might be the reason of the difference in yields of essential oils (Raina et al. 2002).

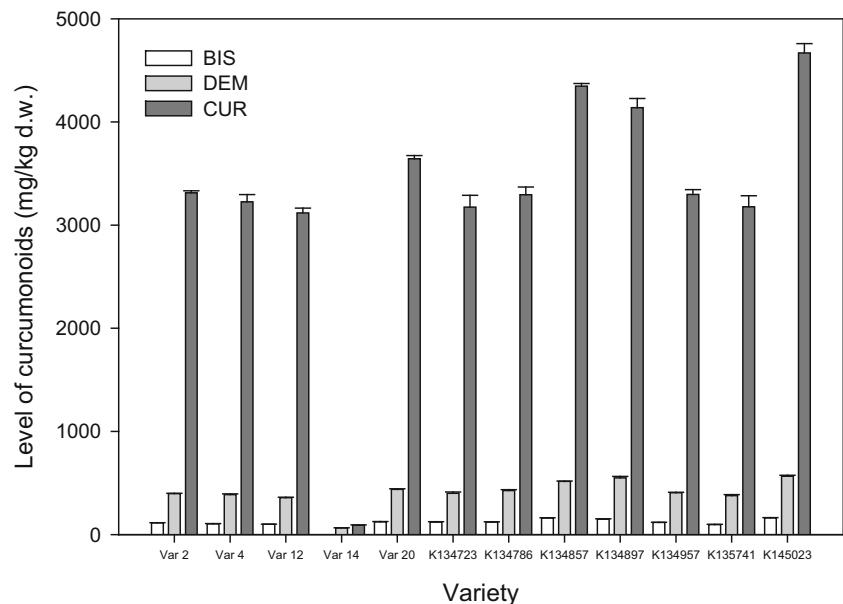
Samples of extracted oil were analyzed to identify the composition by GC-MS. Table 2 shows the substances found in the turmeric essential oil. In total, fourteen compounds were identified in the essential oils. Generally, essential oils showed similar profiles in chemical composition but different contents.

The major components in the essential oils were α -zingiberene (27.70–38.96 %), ar-turmerone (19.54–32.24 %), β -sesquiphellandrene (13.14–18.23 %), α -turmerone (3.72–6.50 %), β -turmerone (2.86–5.60 %), and β -bisabolene (2.50–3.63 %). These findings are in agreement with previous reports in which ar-turmerone, α -turmerone, β -bisabolene, β -turmerone, and β -sesquiphellandrene are the major components (Sharma et al. 1997; Neela et al. 2002; Singh et al. 2003; Jayaprakasha et al. 2005; Raina et al. 2005; Awasthi and Dixit 2009). But, the turmeric grown in Korea contain more α -zingiberene and β -sesquiphellandrene than turmeric grown in India, Bhutan, and Nigeria. The discrepancy in major compounds in the essential oils might be due to the difference in cultivation regions.

The essential oil components are also different from the part of extraction, leaf, flower, or rhizome and processing. The major constituents of turmeric oil from rhizome grown in northern Indian were ar-turmerone (31.7 %), α -turmerone (12.9 %), and β -turmerone (12.0 %), while the oil from leaf contains α -phellandrene (9.1 %), terpinolene (8.8 %), 1,8-cineole (7.3 %), and undecanol (7.1 %) (Awasthi and Dixit 2009). Himalayan region turmeric rhizome oil contains the most amount of α -turmerone (44.1 %), β -turmerone (18.5 %), and ar-turmerone (5.4 %). But, α -phellandrene (53.4 %), terpinolene (11.5 %), and 1,8-cineole (10.5 %) were the major constituents in leaf oil of Indian turmeric (Raina et al. 2002). Nigerian turmeric rhizome oil has β -bisabolene (13.9 %), trans-ocimene, myrcene, 1,8-cineole, α -thujene, and thymol (Kamalirousta et al. 2013).

Table 1 Curcuminoids and essential oil contents in different varieties of turmeric grown in Korea (mg/kg d.w., Values are mean \pm SD, $n = 3$)

Variety	CUR	DEM	BIS	Total	Essential oil content (mg/50 g)
Var 2	3313.1 \pm 19.6	397.8 \pm 2.5	114.1 \pm 0.7	3825.0 \pm 22.7	393.1
Var 4	3225.9 \pm 69.5	387.5 \pm 7.1	104.4 \pm 2.1	3717.8 \pm 78.6	52.9
Var 7	102.9 \pm 1.6	37.0 \pm 0.1	–	140.0 \pm 1.7	–
Var 10	30.6 \pm 0.7	23.3 \pm 0.4	–	53.9 \pm 1.0	–
Var 12	3118.1 \pm 46.3	357.2 \pm 5.2	100.2 \pm 1.2	3575.5 \pm 52.6	250.0
Var 13	–	–	–	–	–
Var 14	91.7 \pm 2.6	64.2 \pm 1.8	–	155.9 \pm 4.3	–
Var 17	35.3 \pm 0.1	20.1 \pm 0.1	–	55.4 \pm 0.3	–
Var 20	3643.6 \pm 30.4	437.7 \pm 5.5	124.0 \pm 1.7	4205.4 \pm 37.6	630.1
K134723	3174.9 \pm 113.6	399.6 \pm 13.5	120.7 \pm 4.6	3695.2 \pm 131.7	227.6
K134786	3294.0 \pm 75.6	426.3 \pm 8.5	120.6 \pm 3.3	3840.9 \pm 87.6	364.6
K134857	4347.1 \pm 25.8 _v	515.9 \pm 3.1	160.8 \pm 1.9	5023.7 \pm 30.9	696.6
K134897	4137.8 \pm 89.9	550.1 \pm 13.0	148.9 \pm 3.6	4836.8 \pm 106.6	676.4
K134956	281.2 \pm 2.0	186.9 \pm 1.4	–	468.1 \pm 3.4	–
K134957	3297.8 \pm 46.1 _v	406.1 \pm 4.4	118.1 \pm 1.7	3822.0 \pm 52.1	472.9
K135741	3177.5 \pm 106.7	376.9 \pm 10.4	96.6 \pm 3.2	3651.0 \pm 120.3	389.5
K145023	4669.7 \pm 89.3	565.4 \pm 9.6	161.0 \pm 2.9	5396.0 \pm 101.8	649.3
KNP050602	–	–	–	–	–

Fig. 3 Curcuminoid amount found in turmeric varieties grown in Korea

The drying process affects the component in oil, where the major components were ar-turmerone (24.4 %), α -turmerone (20.5 %), and β -turmerone (11.1 %) in fresh rhizome oil and ar-turmerone (21.4 %), α -santalene (7.2 %), and ar-curcumene (6.6 %) in dry rhizome oil.

Singh et al. reported that α -Zingiberene (2.5 %), α -turmerone (20.5 %), and β -turmerone (11.1 %) in fresh

rhizome oil were decreased to 0.8, 0.6, and 4.3 %, respectively, during drying (Singh et al. 2003).

Tsai et al. (2011) reported that the major compounds in the essential oils were curcumol (35.77 %) and 1,8-cineole (12.22 %) for *C. aromatica*, and ar-turmerone (49.04 %), humulene oxide (16.59 %), and β -selinene (10.18 %) for *C. longa* (Tsai et al. 2011).

Table 2 Essential oil composition of different varieties of turmeric grown in Korea (area %)

Compound	KI on DB-5	Var 2	Var 4	Var 12	Var 201	K134723	K134786	K134857	K134897	K134957	K135741	K145023
1,8-cineole	1033	0.13	0.17	0.63	0.14	0.15	0.34	0.20	0.15	0.17	0.26	0.22
α -terpinolene	1090	0.09	0.13	0.56	0.10	0.15	0.37	–	0.10	0.20	0.25	0.11
β -caryophyllene	1438	0.69	0.49	0.60	0.59	0.38	0.54	0.47	0.45	0.51	0.60	0.49
γ -elemene	1440	0.48	0.38	0.52	0.43	0.35	0.42	0.43	0.40	0.44	0.44	0.43
γ -curcumene	1486	1.36	1.12	1.14	1.23	1.03	1.26	1.16	1.11	1.36	1.33	1.16
ar-curcumene	1488	2.14	1.65	2.08	1.78	1.28	2.15	1.46	1.39	2.41	1.94	1.62
α -zingiberene	1502	36.75	30.22	38.96	33.56	27.70	31.57	34.43	32.04	35.06	35.87	32.10
β -bisabolene	1515	3.46	2.82	3.63	3.09	2.50	3.01	3.07	2.88	3.39	3.33	3.01
β -sesquiphellandrene	1535	17.45	14.82	18.23	15.83	13.14	15.38	16.22	15.21	16.86	16.57	15.85
β -elemene	1611	1.24	1.48	1.10	1.36	1.71	1.45	1.34	1.39	1.31	1.38	1.47
ar-turmerone	1679	23.50	30.61	19.54	26.97	32.24	28.28	25.22	29.65	21.87	23.90	28.28
β -turmerone	1672	3.82	4.48	2.86	4.41	5.60	4.62	4.01	4.10	4.63	4.60	3.99
α -turmerone	1712	4.00	5.75	3.72	4.85	6.50	4.94	4.92	5.53	4.05	4.13	5.31

Zingiberene is a major constituent in Ginger essential oil (Kamaliroosta et al. 2013), and Ar-turmerone induced neural stem cell proliferation in vitro and in vivo, thus was a candidate to support regeneration in neurologic disease (Hucklenbroich et al. 2014).

Turmeric grown in Korean contain different amounts of curcuminoids and essential oil. From these results, varieties containing much of curcuminoids and essential oil such as K145023, K134857, or K13489, could be selected and cultivated to get source for the health-promoting food.

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