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단 신

울금 추출물의 구성 성분 분석과 *m*-Chloroperoxybenzoic Acid와의 반응

박국태* · 최성목 · 김동진 · 변상욱 · 박용덕 · 강준구 · 조성윤 · 김진명
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Constituent Analysis of Turmeric (*Curcuma longa L*) Extract and Its Reaction with *m*-Chloroperoxybenzoic Acid

Kuk-Tae Park*, Sung-Mok Choi, Dong-Jin Kim, Sang-Wook Byeon, Young-Duk Park, Jun-Gu Kang, Sung-Yoon Cho, and Jin-Myung Kim
Department of Chemistry Education, Korea National University of Education, Chungbuk 363-791, Korea
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Recently many researchers have been trying to extract biologically active materials from natural resources, and to discover natural products that have an excellent efficacy while being nontoxic to human body.[1] Natural products are generally proved to be safe since they have been selected and utilized for a long time as herbal medicines. Since various herbal medicines with antioxidative activity are being utilized in daily life, antioxidative foods or additives are being developed and commercialized.[2] Turmeric is a herbal medicine which has various physiological effects.[3-6]

Turmeric, also called *Curcuma Longa L*, is a perennial herb, which belongs to the Zingiberaceae family and originates from India and Tropical Asia. Turmeric is also called ulgum, gulgum, okgum, and simhwang in Korea. The constituents of turmeric differ according to the area it is grown, but the main constituents are known as curcumin, tumerone, and dicafferoyl methane.[7]

Turmeric is used as a food additive for its antibacterial effect, and it has a high antioxidative activity[8] compared to other herbal medicines.[9] It has also been discovered that *a*-tumerone, which is one of the constituents of turmeric, has an activity of destroying the cell lines of leukemia without side effects.[10] Furthermore, extract from turmeric is used as a natural dye for the yellow color.[11,12]

Many researches have been conducted on the antioxidative activity of turmeric,[13-15] and it has been found out that turmeric prevents aging by eliminating active oxygen. Excessive active oxygen is known to be the cause of ageing and related diseases because it attacks the cells of the human body.[15] Though the physiological effect on some constituents of turmeric is known,[16] it is not known on which constituent acts as an antioxidant. Furthermore, since most of the physiological effects of turmeric are discovered through clinical tests,[8,9] no specific research has been reported on how turmeric acts as an antioxidant.

Therefore, this research was conducted to analyze the constituents and find out the antioxidative activity of the turmeric extract through a kinetic experiment on the reaction of turmeric extract and *m*-chloroperoxybenzoic acid (*m*-CPBA), which is an oxidative reagent with a high reactivity and

stereoselectivity.[17]

EXPERIMENTAL

Materials and measurements. Acetic acid (Showa Chemicals Inc., 99%) and ethanol (Hayman Limited, 99.9%) were used as the solvents in this experiment. *m*-Chloroperoxybenzoic acid (*m*-CPBA, Aldrich Chemical Company, Inc., 57-86%) was purified by literature methods[18] and recrystallized from CH_2Cl_2. The turmeric was purchased from a herbal medicine store. The UV/VIS spectrum was measured on a Hewlett Packard HP 8452A diode-array UV/VIS spectrophotometer. The IR spectrum was measured by Bruker Vector-22 IR spectrophotometer with a KBr pellet. The GC/MS data was obtained by HP-6890 Plus gas chromatography and the mass spectrometer of the HP-5973 MSB in the Hazardous Substance Research Team of Korea Basic Science Institute Seoul Branch. The mass spectrum was analyzed using the mass spectral library search database Wiley 275000.

Preparation of turmeric extract. 1.06 g of turmeric and 130 mL of ethanol were placed in the 250 mL round bottomed flask with a reflux condenser and refluxing it for 33 hours. Ethanol extract was cooled at room temperature. The supernatant of ethanol extract was filtered and evaporated by a rotary vacuum evaporator to give a reddish orange colored turmeric extract. Turmeric extract was dried in a vacuum (below 10 mmHg) desiccator for 12 hours in room temperature, and it used as a reagent.

Preparation of stock solutions. The molecular weight of the turmeric extract was estimated to be about 210, which was calculated by considering the molecular weight and the relative abundance of the constituents from the GC/MS data (*Table* 1). The 1.0×10^{-2} M stock solution of turmeric extract was made by dissolving turmeric extract 0.0027 g (0.01 mmol) in acetic acid and making the amount of the solution 1 mL. The 1.0×10^{-1} M stock solution of *m*-CPBA was made by dissolving *m*-CPBA 0.0173 g (0.1 mmol) in acetic acid and making the amount of the solution 1 mL. Fresh stock solutions were always made before using.

Reaction of turmeric with *m*-CPBA. Reaction of turmeric with *m*-CPBA in acetic acid was monitored from measurements of the decrease in UV/VIS absorbance at 418 nm. UV/VIS absorbances were measured using a 1-cm quartz cell sealed by a rubber stopper. The temperature of the thermostated cell block in UV/VIS spectrophotometer was maintained at a constant temperature (± 0.01 °C) using NESLAB model EX-210 constant temperature bath and circulator. After putting a small magnetic bar in 1-cm quartz cell and flushing it with dry nitrogen, 2.0 mL acetic acid was injected and the cell was sealed with a rubber stopper. Acetic acid in the cell was kept in the thermostated cell block for more than 20 minutes in the desired temperature of the reaction. For the reaction conditions, an appropriate amount of stock solutions was injected at the same time, and the decrease in UV/VIS absorbance was measured at 418 nm as a function of reaction time.

The reaction condition was a pseudo-first-order reaction, in which the concentration of *m*-CPBA was 20 times lager than the concentration of turmeric extract. The kinetic study of the reaction of turmeric extract and *m*-CPBA in the acetic acid was performed by varying the reaction temperature range of 30-50 °C, when the mole ratio of turmeric extract and *m*-CPBA was varied to 1:20, 1:40, 1:80. The pseudo-first-order reaction rate constants were obtained from the slopes of plots of $\ln (A_\infty - A_0)/(A_\infty - A_t)$ vs. time derived from $k = 1/t \ln (a/a - x)$. The values of the activation parameters were obtained by using the Arrhenius plot at three different temperatures and the Eyring equation.[19]

RESULTS AND DISCUSSION

The UV/VIS wavelength maximum of the turmeric extract appeared at 418 nm (ε_{max} 370), because of a long chain conjugation or a poly-nuclear aromatic chromophore. The IR absorption peak of the turmeric extract was shown at 3378 cm^{-1} as a broad band, which corresponds to the

Table 1. Gas chromatogram data and mass spectral data of turmeric extract

Peak	Retention Time, min	Peak height	Relative abundance, %	Molecular weight	Molecular formula
1	9.054	8366	2.780	150	$C_9H_{10}O_2$
2	9.769	5983	0.638	-	-
3	10.510	3985	0.134	-	-
4	10.867	31657	1.792	202	$C_{15}H_{22}$
5	11.356	3527	0.170	-	-
6	12.044	6763	0.574	-	-
7	12.388	13308	1.252	164	$C_{11}H_{16}O$
8	13.156	1288414	80.674	216	$C_{15}H_{20}O$
9	13.672	107897	8.619	218	$C_{15}H_{22}O$
10	14.307	3296	0.280	110	$C_7H_{10}O$
11	14.717	18735	2.349	218	$C_{15}H_{22}O$
12	15.021	1917	0.243	-	-
13	15.312	1577	0.175	-	-
14	15.471	1704	0.322	-	-

hydrogen bonds of -OH bonds of the constituent and ethanol residue. The IR absorption peaks at the 3015-2925 cm^{-1} region correspond to C-H bonds. The IR absorption peaks shown at the 1600-1700 cm^{-1} region correspond to the C=O bonds and C=C bonds, and those at the 1600-1400 cm^{-1} region correspond to the aromatic rings of the aromatic compounds.

The GC/MS data of the turmeric extract is shown in *Table* 1. The gas chromatogram data revealed that there are at least 14 constituents in the turmeric extract. Only 7 compounds could be identified by MS database Wiley 275000, and it was difficult to identify for the other 7 constituents with the GC/MS data alone.[20] Furthermore, the constituents $C_{15}H_{20}O$ and $C_{15}H_{22}O$ which had a retention time of 13.156 min and 13.672 min, respectively constituted 89.29% of the overall abundance (*Table* 1).

Through the mass spectrum and the mass spectral library search database, the molecule structures of 7 constituents of turmeric extract could be identified. They were 2-methoxy-5-cinylphenol ($C_9H_{10}O_2$), 1-(1,5-dimethyl-4-hexenyl)-4-methylbenzene ($C_{15}H_{22}$), 1-(2-methoxy-1-methylethyl)-2-methylbenzene ($C_{11}H_{16}O$), *β*-turmerone ($C_{15}H_{20}O$), *a*-turmerone ($C_{15}H_{22}O$), 2,3,5-trimethylfuran ($C_7H_{10}O$), and *a*-atlantone ($C_{15}H_{22}O$). Among these constituents, *β*-turmerone (*Figure* 1*)* constituted 80.67% abundance (*Table* 1).

In order to perform a kinetic study on the reaction between turmeric extract and *m*-CPBA,

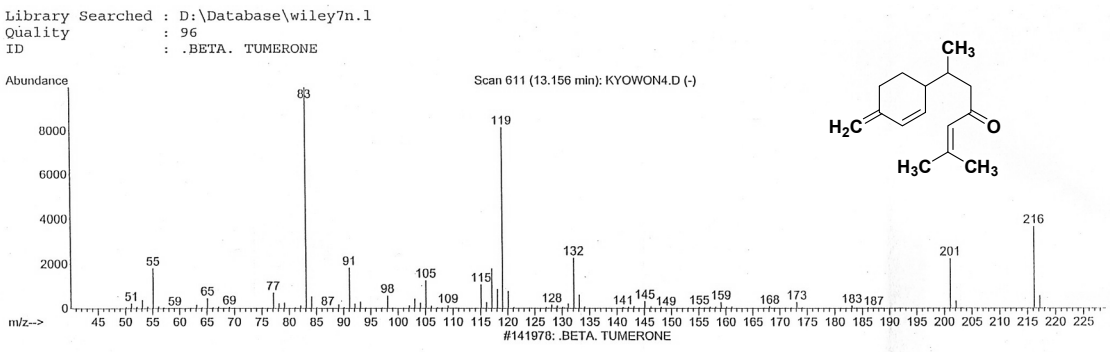

Fig. 1. Mass spectrum and molecular structure of *β*-turmerone which gas chromatogram retention time was 13.156 minute.

Table 2. Pseudo first order reaction rate constants for the reaction of turmeric extracts with *m*-CPBA at mole ratio and temperature in acetic acid

Mole ratio	$k \times 10^4$, sec^{-1}		
	30 °C	40 °C	50 °C
1:20	1.336	2.259	4.331
1:40	2.812	5.396	9.920
1:80	5.180	9.404	17.467

the decrease in UV/VIS absorbance was measured at 418 nm as a function of reaction time, The reaction temperature was 30 °C, 40 °C, and 50 °C when the mole ratio of turmeric extract and *m*-CPBA was 1:20, 1:40, and 1:80, respectively. As a function of reaction time, the absorbance decreased at the 418 nm and increased at the 340 nm, which showed the occurrence of oxidative cleavage reactions. The UV/VIS wavelength maximum of the reaction product shifted to the short wavelength region compared to that of the turmeric extract, which means that the molecular structures of the turmeric extract are cleaved by the reactions. In other words, the π electron conjugation system of the turmeric extract is cleaved by the oxidative reactions.

The pseudo first order reaction rate constants were obtained from the first order reaction rate law by measuring the absorbance at the UV/VIS λ_{max} 418 nm of the turmeric extract, varying the reaction temperature 30 °C, 40 °C, and 50 °C when the mole ratio of the turmeric extract and *m*-CPBA was 1:20, 1:40, and 1:80, respectively (*Table* 2).

The Arrhenius plot of the reaction of turmeric extract and *m*-CPBA in the acetic acid is shown on

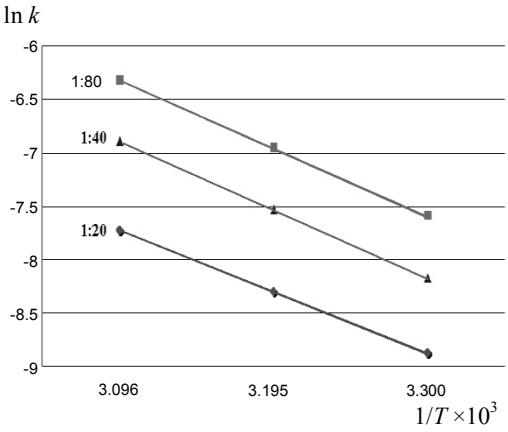

Fig. 2. The Arrhenius plots for the reaction of turmeric extracts with *m*-CPBA at the mole ratio in acetic acid.

Figure 2. Using this plot the activation energy (*Ea*) was found to be 11.46 kcal/mol. 12.27 kcal/mol, 11.83 kcal/mol, when the mole ratio was 1:20, 1:40, and 1:80, respectively. In addition, the thermodynamic activation parameters, which were the activation enthalpy $\triangle H^{\ddagger}$, activation entropy $\triangle S^{\ddagger}$, and the activation free energy $\triangle G^{\ddagger}$, were determined according to the reaction conditions (*Table* 3).

The activation energy was found to be 11.46-12.27 kcal/mol by varying the reaction temperature to 30 °C, 40 °C, and 50 °C when the mole ratios of the turmeric extract and *m*-CPBA in the acetic acid were 1:20, 1:40, and 1:80. The reaction rate constant increased 1.7-1.9 times when the reaction temperature increased 10 °C. The activation entropy was a big negative, -35.89 ~ -40.70 e. u. to the reaction conditions (*Table* 3).

Table 3. Thermodynamic activation parameters for the reaction of turmeric extracts with *m*-CPBA at mole ratio and temperature in acetic acid

Activation parameters	Mole ratio (1:20)			Mole ratio (1:40)			Mole ratio (1:80)		
	30 °C	40 °C	50 °C	30 °C	40 °C	50 °C	30 °C	40 °C	50 °C
$\triangle H^{\ddagger}$, kcal mol^{-1}	10.85	10.83	10.81	11.67	11.65	11.63	11.23	11.21	11.19
$\triangle S^{\ddagger}$, e. u.	-40.47	-40.70	-40.60	-36.29	-36.35	-36.42	-37.04	-36.48	-35.89
$\triangle G^{\ddagger}$, kcal mol^{-1}	23.12	23.57	23.93	22.67	23.03	23.40	22.45	22.63	22.78

Reactions that have an activation energy of below 19 kcal/mol in organic chemical reactions occur spontaneously at room temperature or at below room temperature.[21] Since the oxidation reaction of turmeric extract and *m*-CPBA can occur easily because of the low activation energy of 12.27 kcal/mol, turmeric extract can easily interact with oxidative reagent. This kinetically shows that turmeric extract has an antioxidative activity that can remove highly active oxygen. The activation entropy having a big negative of -35.89 ~ -40.70 e. u., shows that turmeric extract can form a stable transition state with active oxygen, which has a high degree of freedom.[22] Furthermore, this shows that turmeric extract easily reacts with active oxygen and can act as an antioxidant. Whereas the detailed reaction mechanism of the antioxidative activity of the turmeric extract can be examined by a kinetic study of each constituent of the turmeric extract.

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