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Kinetic Studies on the Hydrogenation of Curcuminoids Isolated from *Curcuma Longa* by LC/MS

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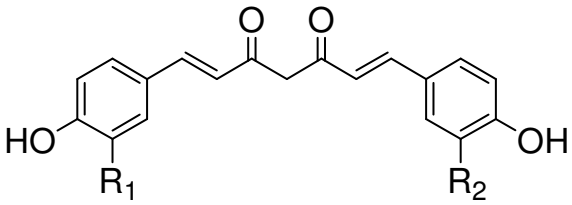
Abstract

In the present study, we investigated the catalytic palladium hydrogenation of curcuminoids, the major constituent isolated from Turmeric root (Curcuma longa) at various time intervals and monitored by LC/MS. The study revealed that the most of the curcuminoids hydrogenated within two hours period and gave the corresponding tetra, hexa and octa-hydrocurcuminoids at different ratio except bisdemethoxycurcumin, which gave only hexa-hydrogenated derivative. Even though, Palladium-carbon catalyst does not favour the reduction of carbonyl group, due to the presence of the conjugated double bonds hydrogenation occurred.

Keywords: Hydrogenation, curcumin, hexahydrocurcumin, tetrahydrocurcumin.

Introduction

Curcumin, extracted from the dried turmeric root of plant *Curcuma longa* by solvent extraction and the major active constituent in turmeric which is commonly used for health care and as food ingredients. Turmeric has a long history of use in Ayurvedic medicine for the treatment of inflammatory conditions[1] and a wide variety of diseases including those of the skin, pulmonary, and gastrointestinal systems, aches, pains, wounds, sprains, and liver disorders[2]. Turmeric constituents include the three curcuminoids namely, Curcumin (C1), Demethoxycurcumin (C2), and Bisdemethoxycurcumin (C3). The two double bonds conjugated with beta-diketones play the main role to give the characteristic colour to the curcumin. The chemical structures of the three curcuminoids are shown in figure-1. Apart from these pharmacological properties, the bio-availability of the total curcuminoids in the animal systems is far less[3].



R_1, R_2 = OMe, Curcumin (C1), R_1 = OMe, R_2 = H, Demethoxycurcumin (C2), R_1, R_2 = H, Bisdemethoxycurcumin (C3)

Figure 1
Different curcuminoids present in Turmeric

The double bonds of curcumiods can be hydrogenated by using catalyst[4,5] and the obtained product is light yellow to brown having more bio-availability than the curcuminoids. These are the main metabolite of curcumin namely, Tetrahydrocurcumin, (THC), Hexahydrocurcumin (HHC) and Octahydrocurcumin (OHC) and showed greater DPPH (2,2-diphenyl-1-picrylhydrazyl) radical trapping activity, inhibition of linoleic acid peroxidation and free radical induced red blood cell hemolysis than their curcumin parent compounds[6]. Tetrahydrocurcuminoids show more anti-oxidative activity than the parent curcuminoids[7] and act against ferric nitrilotriacetate (Fe-NTA)-induced oxidative renal damage were studied in male mice[8]. Another study indicates that THC exerts more protection than curcumin against chloroquine (CQ, a drug to prevent Malaria) induced toxicity by its ability to improve the lipid peroxidation through the free radicals scavenging activity, which further enhanced the levels of antioxidant defence system in the liver and plasma[9]. Hexahydrocurcumin (HHC) has been demonstrated to have significant larvicidal activity against some parasitic species *in vitro* and *in vivo*[10] Khanitta Srimuangwong *et* al revealed that HHC together with 5-FU exerts a synergistic effect and prove chemotherapeutically useful in treating human colon cancer[11]. Herein we first report, to the best of our knowledge, a study of hydrogenation of curcuminoids at various time intervals by the aid of LC-MS and HPLC methods.

Material and Methods

The curcumin used for the hydrogenation is obtained in house by solvent extraction. Purity of the Curcminoids determined by HPLC[12] (C1=80.29%, C2=15.53%, C3=2.31%). Palladium on carbon and Ethyl acetate purchased from Spectrochem PVT LTD, Mumbai, India. All solvents used for the LC/MS analysis obtained from Merck.

General procedure: Hydrogenation was carried out by dissolving 50g of curcumin in 1L of ethyl acetate and added 2.5g of 10% Palladium on carbon to it in an autoclave.

Degassed two times and maintained 2Kg pressure of hydrogen at 50^0C. During the hydrogenation, the samples were withdrawn at 2, 6, 8, 14 and 20 hours time periods. After concentrated off the solvent, samples were analyzed by LC/MS.

Detection Method: The amount of curcumin remaining in the reaction mixture was determined by HPLC at different intervals as follows:

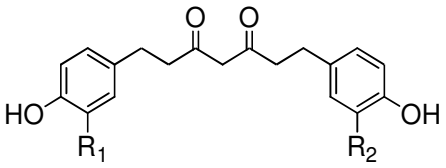
A reverse-phase HPLC system developed was used to determine the progress of the reaction. It consisted of a pump (Waters 515 HPLC Pump), UV/visible detector (wavelength 420 nm, Waters 2489 UV/visible detector) and a column (Xterra 5 µm C18, 250×4.6mm diameter, Waters). The isocratic mobile phase was 60% water containing 1% citric acid and 40% THF and a flow rate maintained at 1mL/min. 10 µL of sample were injected each time and run time was 30min. Empower 3 software (Waters) was used to record chromatograms and check the curcuminoids peak diminishing.

A reverse-phase gradient LC/MS method was used to separate, identify and quantify the hydrogenated curcuminoids. Agilent 1260 infinity quaternary gradient HPLC system consisting of auto sampler with Eclipse Plus C18 column, 5µ (250 x 4.6 mm), was used to obtain the chromatograms and Agilent 6120 Quadrupole LCMS was used to obtain the MS spectra, performed in negative electrospray ionization (ESI) mode. 0.05% acetic acid in Nano pure water (A), 0.05% acetic acid in acetonitrile (B), 1% ammonium acetate (C), and 0.05% acetic acid in methanol (D) were used as mobile phases and the flow rate was 1.0 mL/min. The mobile phases used as in the following manner: At 0-4 min, 2% B and 98% C; 4-10 min, 40% B and 60% C; 10-11 min, 100% C; 11-14 min, 40% C and 60% D; 14-15 min, 60% A and 40% B; 15-32 min, 40% A and 60% B and at 32-47 min, 100% B. Nitrogen was used as a drying gas at a flow rate of 600 L/hour, the drying gas temperature was 350 ˚C and the ion spray voltage was 4000V.

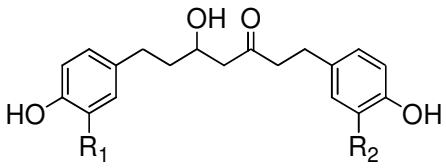
Results and Discussion

The main constituents formed during the hydrogenation reaction are Tetrahydrocurcumin (THC1), Hexahydrocurcumin (HHC1), Octahydrocurcumin (OHC1), Tetrahydrodemethoxycurcumin (THC2), Hexahydrodemethoxycurcumin (HHC2), Octahydrodemethoxycurcumin (OHC2), Tetrahydrobisdemethoxycurcumin (THC3), Hexahydrobisdemethoxycurcumin (HHC3) and Octahydrobisdemethoxycurcumin (OHC3) and the chemical structures are as in the figure-2.

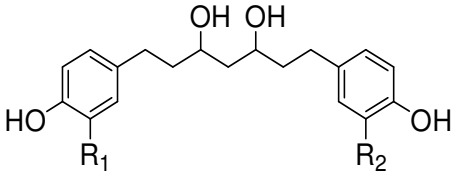
Apart from these there are some fragmented molecules also found in LC/MS. The chemical structures are as in the figure-3.



R_1, R_2 = OMe, THC1, R_1 = OMe, R_2 = H, THC2, R_1, R_2 = H, THC3

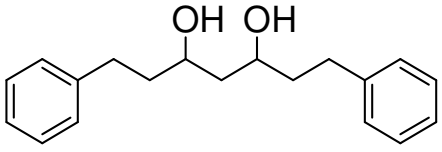


R_1, R_2 = OMe, HHC1, R_1 = OMe, R_2 = H, HHC2 R_1, R_2 = H, HHC3

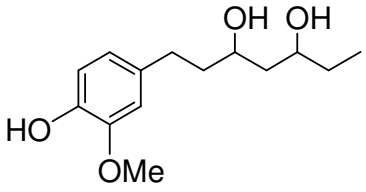


R_1, R_2 = OMe, OHC1, R_1 = OMe, R_2 = H, OHC2, R_1, R_2 = H, OHC3

Figure 2
Hydrogenated curcuminoids formed during the reaction



m/z =284.1



m/z = 254.1

Figure 3
Fragmented molecules formed in the reaction

The most of the curcuminiods (C1, C2 and C3) were hydrogenated within 2 hrs period and THC1 (47.88%), HHC1 (6.51%), THC2 (15.55%) and OHC1 (2.78%) formed prominently as in the table-1 and as in the LC/MS spectra, figure-4. Generally, Palladium-carbon catalyst does not favour the reduction of carbonyl group, but due to the presence of the conjugated double bonds with the carbonyl groups, hydrogenation happened. Around 1% C1 remained and C2 and C3 consumed almost as in the data and this is graphically represented in figure-5. After 6 hours of the reaction, THC1 content increased to 51.48%, whereas other hydrogenated curcuminoids did not increase considerably. At 8th hours of the reaction, the THC1 composition didn't increase noticeably and remained as same as in the 6th hour. All other curcuminoids too show the same trend. At 14th hour the THC1 increased to 54.21%, attained maximum conversion and remained same thereafter. The concentration of the other hydrogenated curcuminoids HHC1 (7.88%), THC2 (16.2%), HHC2 (3.07%), OHC2 (1.09%) and HHC3 (0.95%) increased slightly at 14th hour. At 20th hour there is a slight decrease in HHC1 (7.59%), THC2 (16.14%) and HHC2 (2.81%) and for other hydrogenated curcuminoids, the concentration is same as demonstrated in the graph, figure-6. From these data, it is clear that the C3 is converted HHC3 up to 1% and there is no other hydrogenated C3 curcuminoids (THC3 and OHC3). The molecule with m/z 284 showed constantly 2.5% all the time and m/z 254 too showed around 5%.

Table 1
Concentration of curcuminoids at different time intervals (All values in %)

	C1	C2	C3	THC1	HHC1	OHC1	THC2	HHC2	OHC2	THC3	HHC3	OHC3
2hrs	0.99	0.072	0.0045	47.88	6.51	2.78	15.55	2.89	0.94	-	0.88	-
6hrs	0.16	0.016	-	51.48	7.44	2.88	15.59	2.80	0.95	-	0.94	-
8hrs	0.077	0.0072	-	50.84	7.24	3.92	15.76	2.93	1.01	-	0.91	-
14hrs	0.034	-	-	54.21	7.88	4.02	16.20	3.07	1.09	-	0.95	-
20hrs	0.029	-	-	54.45	7.59	4.03	16.14	2.81	1.09	-	0.95	-

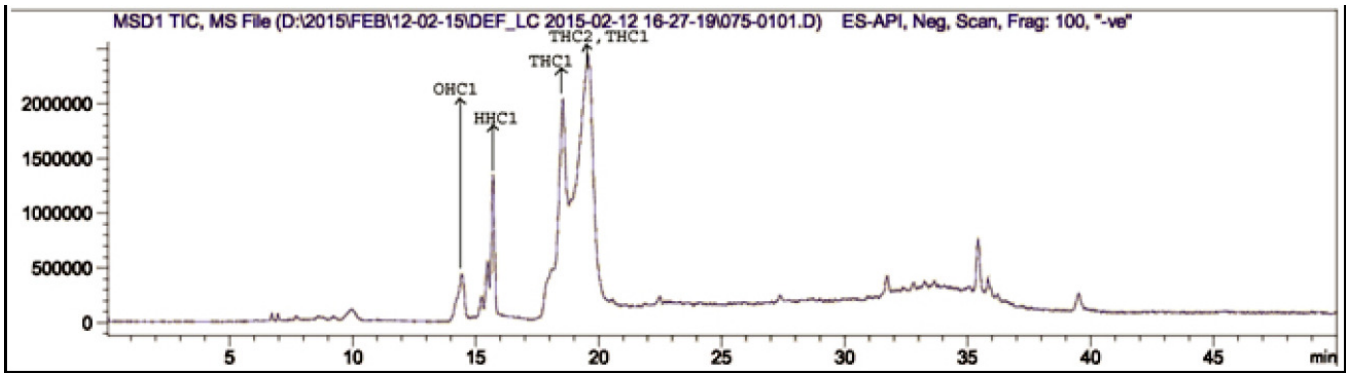


Figure-4
LC/MS spectra of hydrogenated curcuminoids after 20 hours

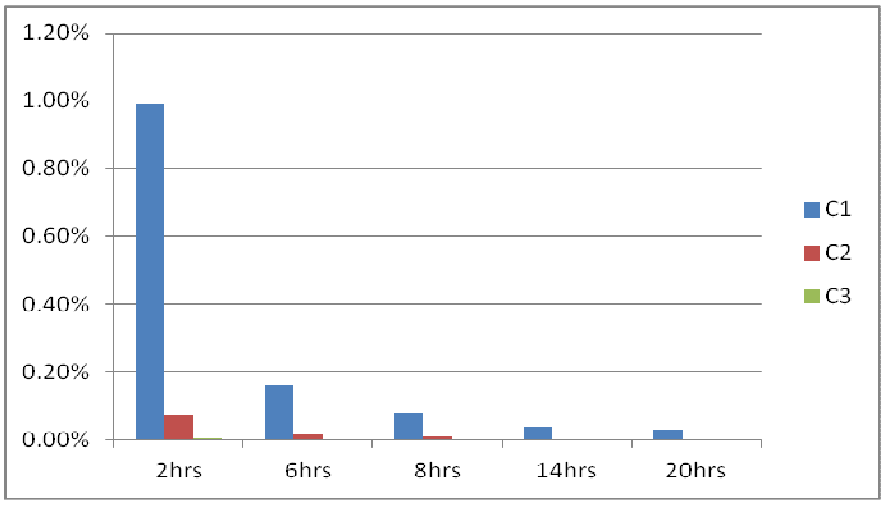


Figure 5
The concentration of C1, C2 and C3 at different time intervals

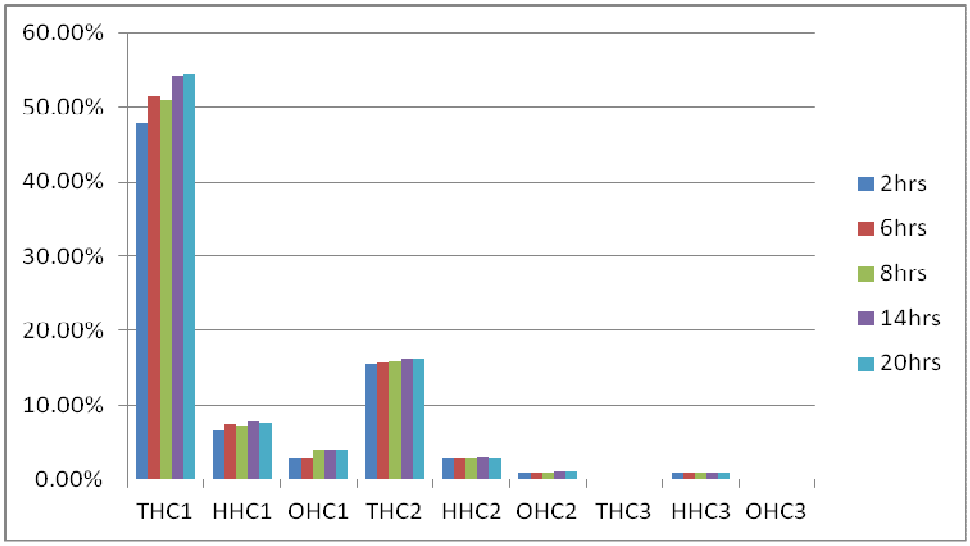


Figure 6
The concentration of different hydrogenated curcuminoids at various time intervals

Conclusion

The study reveals the rate of hydrogenation of curcuminoids at various time intervals and the constituents formed in the reaction analysed by LC/MS. It is clear from the data that most of the curcuminoids hydrogenated within two hours period and gave the corresponding tetra, hexa and octa-hydrocurcuminoids at different ratio except bisdemethoxycurcumin, which gave only hexa hydrogenated derivative. Though, Palladium-carbon catalyst does not favour the reduction of carbonyl group, due to the presence of the conjugated double bonds hydrogenation happened.

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