

A Unique Formulation of Hydrogenated Curcuminoids with Higher Bio-Availability and the Application in Food Matrices

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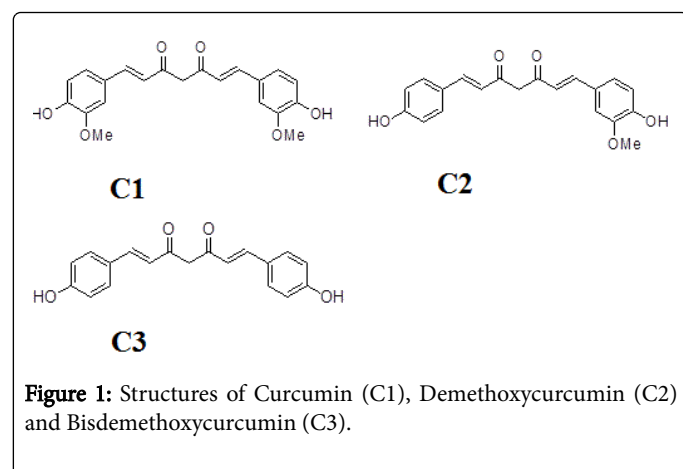
Abstract

Hydrogenation of curcuminoids leads to a unique combination of bio active molecules and the encapsulation of the same with beta-cyclodextrin gives a higher bioequivalency than that of curcumin in animal blood plasma. A bioequivalence study of the newly developed colorless, tasteless curcumin derivative had conducted in female Sprague Dawley rats and the blood serum was analyzed by HPLC. The relative bioavailability (C_{max}) of white curcumin was significantly enhanced to about 6.76 fold (576.206%) compared with that of curcumin 95% in rat when administered the test item and reference standard through oral route. The stability of the white curcumin at normal cooking temperature (120°C - 200°C) is appreciable and there is no considerable degradation in experiments before and after cooking. As this formulation doesn't change the taste and color of the food products, it could be easily used for increasing the nutraceutical benefits.

Keywords: Curcumin; Hydrogenated curcuminoids; Bioequivalence; Rats; Heat stability

Introduction

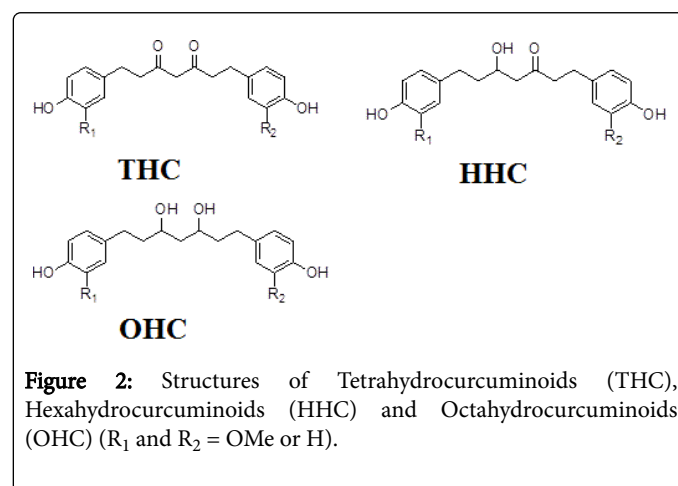
Curcumin, a yellow colored powder, chemically a polyphenol, derived from the rhizome of the herb *Curcuma longa* has a wide range of uses in food, cosmetics and in pharmacology. Chemically, curcumin is called diferuloylmethane, which exhibits keto-enol tautomerism due to its alpha hydrogen [1]. Turmeric has a long history of use in Ayurvedic medicine for the treatment of inflammatory conditions [2], skin whitening and a wide variety of diseases including those of the pulmonary and gastrointestinal systems, aches, pains, wounds, sprains, and liver disorders [3]. Turmeric constituents include the three curcuminoids [4] namely, Curcumin (C1), Demethoxycurcumin (C2), and Bisdemethoxycurcumin (C3) as shown in the Figure 1.



The two double bonds conjugated with beta-diketones usually play the main role to give the characteristic colour to the curcumin. Even

though, curcumin has more pharmacological properties; the amount of total curcuminoids absorbed by the animal systems is far less [5].

Curcumin can be hydrogenated in presence of a catalyst like palladium to obtain its saturated product. The obtained product (hereafter the product is mentioned as white curcumin) contains various chemical components mainly Tetrahydro curcuminoids (THC, 70% - 80%), Hexahydrocurcuminoids (HHC, 10% - 20%) and Octahydrocurcuminoids (OHC, 3% - 5%) as in the Figure 2 [6].



The obtained white curcumin is off white to brown in colour and was encapsulated with beta-cyclodextrin which is a group of seven α -D-glucopyranoside aligned in a cyclic way with a hydrophilic outer surface and a lipophilic central cavity. β -cyclodextrins generally do not permeate lipophilic membranes due to its large size and a number of hydrogen donors and acceptors [7]. Cyclodextrins act as 'molecular cages' [8] which trap the targeted molecules inside the lipophilic cavity and have a wide variety of uses in the pharmaceutical, food and cosmetic industries. Hence, beta-cyclodextrin is used as complexing

agents to increase the aqueous solubility of poorly soluble white curcumin and to increase their bioavailability and stability [9]. The white curcuminoid molecules can be entrapped in cyclodextrin lipophilic cage, hence the white curcumin-cyclodextrin matrix have more bio-availability, solubility and stability [10].

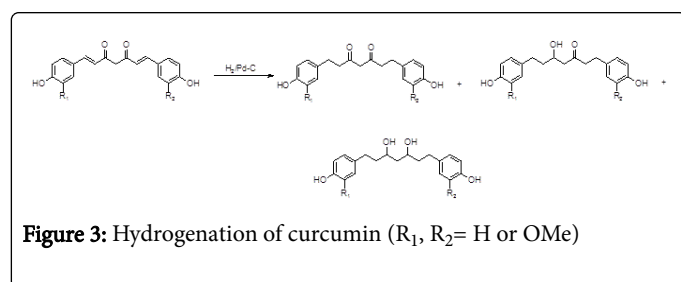
Studies show that the inhibitory activities of tetrahydrocurcumin (THC) on the lipid peroxidation of erythrocyte membrane induced by tert-butylhydroperoxide led a greater effect than curcumin. Li et al. [11] examined the *in vitro* antioxidant and anti-inflammatory activities of hexahydrocurcumin, found that the relative antioxidant potency is greater than that of 6-shogaol and 6-dehydroshogaol [12]. The hydrogenated curcuminoids tetrahydrocurcumin, hexahydrocurcumin and octahydrocurcumin 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. Srimuangwong et al. [13] illustrates that hexahydrocurcumin is a specific COX-2 inhibitor that plays an important role in carcinogenesis and the addition of hexahydrocurcumin to 5-Fluorouracil in HT-29 human colon cancer cells can enhance the growth inhibition and down-regulation of COX-2 [14].

Materials and Methods

The curcumin used is obtained in house by solvent extraction of dried turmeric powder followed by crystallization and the white curcumin prepared by hydrogenation of curcumin as described [15] below. Purity of the curcuminoids determined by HPLC [16] (C1 = 80.29%, C2 = 15.53%, C3 = 2.31%). All solvents used for the HPLC analysis obtained from Merck. The area-under-the-curve (AUC) for the HPLC plot of the plasma concentration over time was calculated using linear trapezoidal method. Graphical representation and C_{max} determined by Graph Pad Prism-6 software.

Hydrogenation of curcumin

The scheme of the hydrogenation of curcumin as shown below (Figure 3):



Suspended 10% Pd/C (5% w/w) in Ethyl acetate (20V) and added Curcumin to it. Hydrogenated at 2 bar pressure in an autoclave at 50°C for 16 h. Reaction was monitored by TLC. After completion of the reaction, reaction mass was filtered and concentrated. The oily matter solidified by overnight. The obtained solid was high sheared in high-shear mixer (Primix T.K. homomixer mark 2) with beta-cyclodextrin (1:3 by w/w) in water (4v) at 70°C and spray dried (Ohkawara Kakohki Co Ltd, Japan, Model L-8) to obtain off-white solid.

Animal subjects and study design

Female Sprague Dawley rats having body weight 150 g – 170 g and 8 - 9 weeks old used for this study were housed in Liveon Biolabs PVT LTD, Tumkur, and Karnataka, India. Animals were housed under standard laboratory conditions, air-conditioned with adequate fresh air supply (Air changes 12 - 15 per h), room temperature 21.3°C to 24.7°C, relative humidity 46% - 68%, with 12 h light and 12 h dark cycle. The temperature and relative humidity was recorded once daily. The experiment conducted as per the recommendation of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for laboratory animal facility and approved by Institutional Animal Ethics Committee (IAEC) protocol.

The study was conducted in three groups consisting of 39 animals. Animal groups viz., G1, G2 and G3, consisting of 9, 15 and 15 animals respectively.

Blood sampling have done as in the given Table 1.

Group	Treatment	Animal Numbers	Blood Sampling Time Points		
G1	Vehicle Control	1-3	Pre Dose	2 h	10 h
		4-6	30 min	3 h	12 h
		7-9	1 h	6 h	24 h
G2	White curcumin	10-14	Pre Dose	2 h	10 h
		15-19	30 min	3 h	12 h
		20-24	1 h	6 h	24 h
G3	Curcumin 95%	25-29	Pre Dose	2 h	10 h
		30-34	30 min	3 h	12 h
		35-39	1 h	6 h	24 h

Table 1: Blood sampling of different groups of rats at different time intervals.

G1 served as vehicle control, G2 treated with white curcumin 25% (25 mg/kg) and G3 treated with curcumin 95% (600 mg/kg) at single dose with a dose volume of 10 mL/kg body weight after overnight fasting. The test items were suspended in distilled water and were administered orally to each animal in a single dose by using gavage needle.

Blood samples were collected from retro-orbital puncture method in 2 mL eppendorf tubes containing 2% K_2EDTA from the animals, at different time points. Plasma was separated, frozen and stored at -20°C.

Sample preparation

Each sample of plasma was taken and allowed to attain room temperature and was extracted with ethyl acetate (3 mL). The extracts of each sample were filtered and pipette out 1 mL into evaporation tubes. The solvent was evaporated under the stream of nitrogen gas, under fume hood. Added 2 mL of methanol to re-dissolve the dried sample and analyzed using HPLC.

HPLC method for white curcumin

The isocratic reagent was prepared by mixing 35% acetonitrile, 41% de-ionized water, 24% methanol and 0.3% TFA (v/v/v/v). The mixed reagent was vacuum filtered and degassed. The separated White curcumin plasma was quantified by HPLC using UV / visible detector (wavelength 280 nm, Waters 2489 UV / visible detector). Samples were injected onto a reversed-phase column (Xterra 5 µm C18, 250 × 4.6 mm diameter, Waters) with a flow rate 1.0 mL/min and the run time was 15 min.

HPLC method for curcumin

A reverse-phase HPLC system developed was used to analyze the blood plasma samples. 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 × 4.6 mm diameter, Waters). The isocratic mobile phase was 40% THF and 60% water containing 1% citric acid, pH adjusted to 3.0 using concentrated potassium hydroxide solution and a flow rate maintained at 1 mL/min. 10 µL of sample were injected each time and run time was 30min. Empower 3 software (Waters) was used to record chromatograms.

Following formulas were used for the calculation of percentage and difference.

Percentage = (Mean of Whitecurcumin 25% concentration - Mean of Curcumun 95% concentration) / Mean of Curcumin 95% concentration X100

Fold difference = (Mean of White curcumin 25% concentration / Mean of Curcumin 95% concentration).

Bread making

The ingredients used for bread making are flour, salt, sugar, yeast and oil. Accurately weighed ingredients are mixed together using a dough machine (Kitchen aid). 0.25% or 0.3% of white curcumin is added along with other ingredients and blended properly. The bread dough is placed in a tray for fermentation for 2 h at room temperature. The dough was then divided into similar weighed pieces. The baking was performed at 180°C in an electric oven (Siemens, HB86K575IN) for 20 min. After baking the bread was taken out and allowed to cool for 1 h at room temperature.

Cookie making

Cookies are small, flat, baked foods. The basic ingredients of cookies are flour, butter, sugar, egg or egg powder. Accurately weighed ingredients are mixed properly using an electric hand Wisk. 0.25% or 0.3% of white curcumin is added along with the other ingredients to make the soft dough. The rolled dough is divided in to equally weighed pieces with 2 cm thickness. The baking was performed at 180°C in an electric oven (Siemens, HB86K575IN) for 10 min. After baking the cookies taken out and allowed to cool at room temperature for 30 min.

Cake making

Cake is a form of sweet desert. Basic cake ingredients are flour, egg, butter, sugar and some leavening agent. 0.25% or 0.3% of white curcumin is weighed along with the flour. The flour, baking powder and melted butter is added to the uniformly mixed sugar and egg. The cake mix is then baked at 200°C in an electric oven (Siemens,

HB86K575IN) for 40 min. After baking the cookies taken out and allowed to cool at room temperature for 2 h.

Sample preparation for HPLC

Accurately weighed the crushed sample (of bread, cake and Cookie) and refluxed in 80 mL HPLC grade methanol at 70°C, for 3 h. Filtered through Whatman No.1 filter paper into a 100 mL standard flask and made up to the mark using methanol. An aliquot is taken and filtered through 0.25 µm Nylon filter and injected.

Results and Discussion

The relative absorption of curcumin and white curcumin in plasma is given in the Table 2.

Time (Hours)	Concentration (µg/mL)		
	Vehicle control	Curcumin 95%	White curcumin 25%
0.5	0	0.2064	0.5455
1	0	0.1346	0.504
2	0	0.2771	0.743
3	0	0.0834	0.7136
6	0	0.0067	0.7491
10	0	0	0.5995
12	0	0	0.4925
24	0	0	0.4417

Table 2: Absorption of curcumin 95% and white curcumin in blood plasma at different time points.

The poor bioavailability of curcumin is mainly because of the poor absorption of the curcumin in the gastrointestinal tract. Once it absorbed, it will convert to the different form of metabolites and the biological action is further increased. White curcumin is a unique blend of major metabolites of curcumin and the encapsulation of the same with beta-cyclodextrin improves the water solubility, so the excretion could be minimalized. White curcumin enhances the absorption to the blood and avoid the further metabolism.

It is clear from the table that the absorption of curcumin is maximum at 2nd h whereas for white curcumin, this is at the 6th h. In the current study, the novel beta-cyclodextrin encapsulated white curcumin was developed and conducted a bioequivalence comparative study of the white curcumin (25 mg/kg body weight) and the curcumin 95% (600 mg/kg body weight). The C_{max} observed is 0.2771 and 0.7491 for curcumin and white curcumin respectively. It is evident that the relative bioavailability of white curcumin was significantly enhanced to about 6.76 fold (576.206%) compared with that of curcumin 95% in rat when administered the test item and reference standard through oral route. The oral bioavailability of encapsulated white curcumin can be improved by increase in the water solubility, the release rate in the intestinal gland, the permeability and the residence time in the jejunum. Thus, encapsulating white curcumin on beta-cyclodextrin is a promising method for sustained and controlled absorption with improved bioavailability. The above data summarized in the graph below (Figure 4):

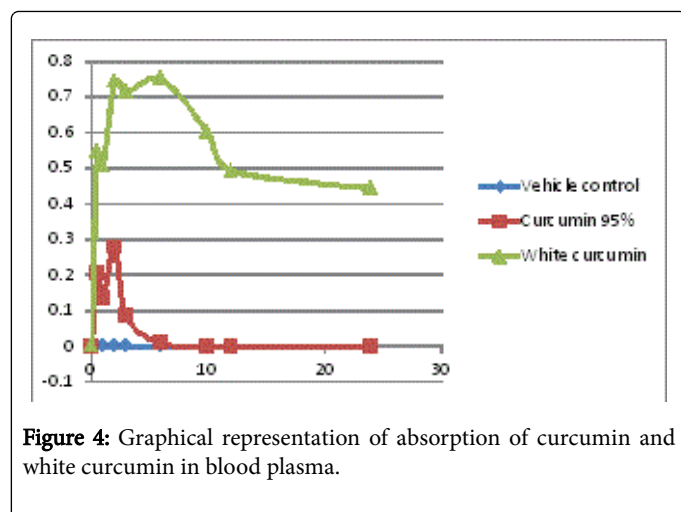


Figure 4: Graphical representation of absorption of curcumin and white curcumin in blood plasma.

Stability study

The heat stability of the white curcumin examined in different food samples and proved that the stability of the white curcumin at normal cooking temperature (120°C - 200°C) is appreciable. The recommended dosage was 0.25% - 0.3% and the results are given below. There is no considerable degradation in experiments before and after cooking, as in the Tables 3 and 4.

Sample		After Blend %	After Cooking %
Bread (180°C)		0.061	0.06
Cake (200°C)		0.059	0.055
Cookies	160°C - 12 min	0.0618	0.0613
	180°C - 15 min	0.0615	0.061

Table 3: Sample 1 white Curcumin 0.25%, dosage (0.0625% actual WC content).

Sensory evaluation

Sensory panel members are requested to compare the organoleptic qualities of white curcumin incorporated bread, cake and cookie with the standard bread, cake and cookie. Organoleptic characters of interest include taste, flavor, color and texture. Sensory analysis results showed that white curcumin does not have any effects with the flavor, taste, color and texture of bread, cake and cookie.

Sample		After Blend %	After Cooking %
Bread (180°C)		0.074	0.072
Cake (200°C)		0.073	0.07
Cookies	160°C - 12 min	0.07	0.07
	180°C - 15 min	0.069	0.068

Table 4: Sample 2 - White curcumin - 0.3% dosage (0.076% Actual WC content).

In summary, the bioequivalence of the newly developed white curcumin in blood serum is prominently higher than that of curcumin 95% and the stability of the white curcumin in different food matrices is ascertainable, hence, it could be easily used for increasing the nutraceutical benefits for different food materials at low to high temperatures.

Supporting Information

HPLC chromatograms and organoleptic images of the food matrices.

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