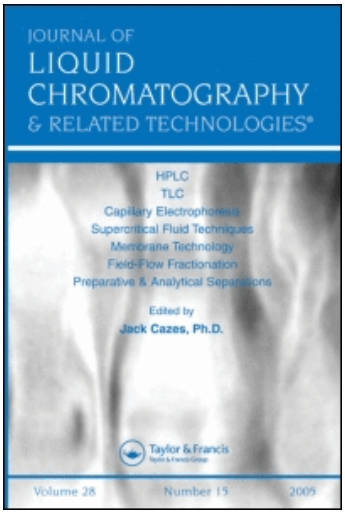

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High Performance Liquid Chromatographic Analysis of Curcuminoids and Their Photo-oxidative Decomposition Compounds in Curcuma Longa L

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF CURCUMINOIDS AND THEIR PHOTO-OXIDATIVE DECOMPOSITION COMPOUNDS IN CURCUMA LONGA L

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ABSTRACT

Photochemical oxidation of curcuminoids such as curcumin, bis-demethoxycurcumin and demethoxycurcumin in dry powder of Curcuma longa L. (zingiberaceae) root and in ethanolic and methanolic extract has been studied. Whatman PartiSphere-5 NH_2 and Whatman PartiSphere-5 WCX columns were used to analyze curcuminoids and their degradation products. The curcuminoids were found to be more stable in the dry powder of Curcuma longa L. root than in ethanolic and methanolic extracts. Vanillin, p-hydroxybenzaldehyde, ferulic aldehyde, p-hydroxybenzoic acid, vanillic acid and ferulic acid were identified as the oxidation products.

INTRODUCTION

The rhizomes of Curcuma longa L. (Zingiberaceae) or curry root have been reported to be the principal source of curcumin [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] and its isomers demethoxy curcumin [1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione] and bisdemethoxycurcumin [1,7-bis-(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione]. Curcuminoids are used as coloring agents in food, drug and cosmetics, and are also regarded as a drug/drug model, as a bile secretion stimulating, hypocholesteremic and antiheptotoxic agent. Curcumin has a poor light stability. Curcumin colored products fade noticeably within the shelf life of the product when no precautions are taken (3). It has been shown to decompose when exposed to UV/visible light, both in solution and as a solid. Vanillin, vanillic acid, ferulic aldehyde and ferulic acid have been identified as the degradation products of the synthetic curcumin (7).

The color reaction with boric acid or the direct spectrophotometric method is the official test for curcumin (4). Separation of curcuminoids usually is accomplished by thin layer or paper chromatography, often in combination with spectroscopy for quantitation. A few HPLC systems based on C-18 stationary phases have been published for analyzing curcumin (1). These chromatographic systems, however, are limited to separating the three curcuminoids. These curcuminoids could not be completely resolved by these systems. Tonneson and Karlsen (5, 7) achieved separation of the curcuminoids on Nucleosil-NH_2 and used Spherisorb RP-18 and Nucleosil-NH_2 columns to analyze synthetic curcumin and its photochemical decomposition products. Nucleosil-NH_2 retained vanillic acid and ferulic acid

where as the RP-18 system allowed their resolution. The curcumin failed to show on the RP-18 column while it could be easily detected in a Nucleosil-NH_2 system. However, the photochemical decomposition of isomers of curcumin such as demothoxycurcumin and bis-demothoxycurcumin in food systems has not been reported.

The present paper reports HPLC analysis of curcuminoids and their photochemical oxidation products in dry powder of Curcuma longa L. and its ethanolic and methanolic extracts on Whatman PartiSphere-5 WCX and Whatman PartiSphere-5 NH_2 columns.

EXPERIMENTAL

Materials

4-Hydroxybenzaldehyde, ferulic acid (4-hydroxy-3-methoxycinnamic acid), curcumin, vanillin (4-hydroxy-3-methoxybenzaldehyde) and vanillic acid were purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI). p-Hydroxybenzoic acid was obtained from Sigma Chemical Company (St. Louis, MO). o-Nitroaniline was supplied by Fluka Chemical Company (Hauppayge, NY). Curcuminoids were isolated and collected from Whatman PartiSphere-NH_2 column by using ethanolic extract of Curcuma longa L. according to the condition described by Tonnesen and Karlsen (5). Hexane, ethanol and methanol were purchased from E.M. Chemicals (Cherry Hill, NJ). Dry powder of Curcuma longa L. root was purchased from Touch of Asia (Hamilton, NJ).

Packing Material

Partisphere-5 WCX, Partisphere-5 NH_2 and PartiSphere-5 C-18 cartridge columns for chromatography were obtained from Whatman Inc. (Clifton NJ).

Sample Preparation

The extracts of dry powder or root of Curcuma longa L. were prepared according to conditions described by Tonnesen et al (6). One or two grams of the dry powder from crushed root were dispersed in 3 to 5 ml of ethanol or methanol, stirred for 15 minutes and then centrifuged.

HPLC Analysis

HPLC was performed using a variable wavelength UV detector, Spectroflow monitor SF-770 (Kratos Analytical, Ramsey, NJ); a programmable solvent delivery system, Series 3B (Perkin-Elmer Corp., Norwalk, Conn.); a manual injection valve, with 50 µl loop (Valco Instruments Co., Houston, TX) and a chart recorder (Laboratory Data Control, Riviera Beach, FL).

Curcumin and their photodegradation products from dry powder of root of Curcuma longa L. were analyzed on Whatman PartiSphere-5 WCX column using o-nitroaniline as the internal standard. The computer analysis of the individual curcuminoids in the mixtures was performed on Whatman PartiSphere-5 NH_2 using weight against area calibration curves.

A hexane:ethanol:water (300:30:0.75) mixture was used as the mobile phase while performing analysis on PartiSphere-5 WCX column. A flow rate of 0.4 ml/min was used in this case. In the case of analysis of curcuminoids on PartiSphere-5 NH_2 column, a mixture containing ethanol:water (96:4, v/v) was used as a mobile phase and a flow rate of 1 ml/min was maintained during the run.

RESULTS AND DISCUSSION

Figure 1 and Table I represent the analysis of ethanolic extract of the dry powder of Curcuma longa L.

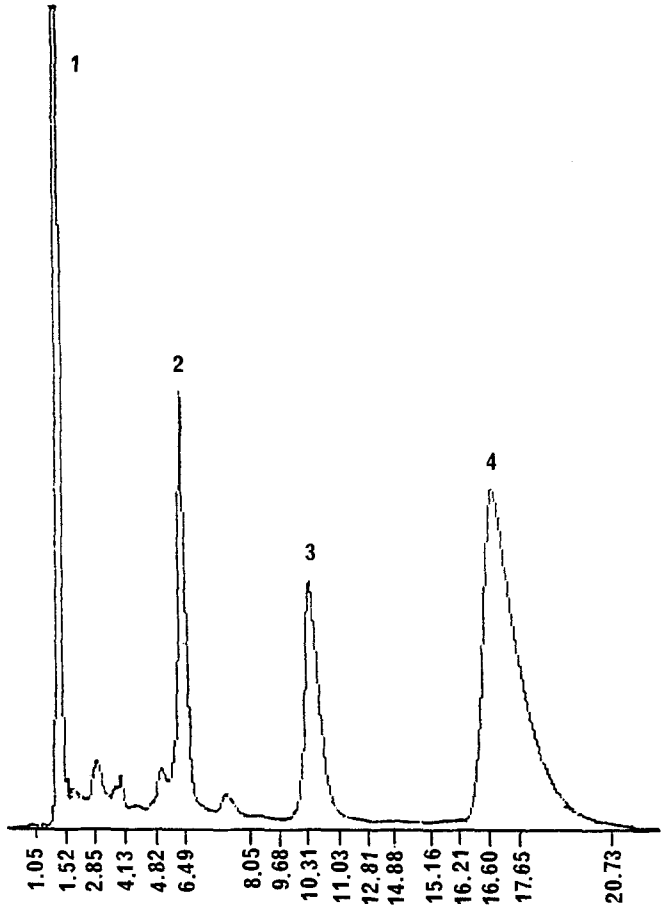

Fig. 1. 1(unknown), 2(bisdemethoxycurcumin), 3(demethoxycurcumin) and 4(curcumin). Mobile phase: ethanol : water (96:4, v/v); Flow rate: 1 ml/min. λ_{max}: 280 nm; Sample volume injected: 20 µl; Sample description: ethanolic extract of dry powder of Curcuma longa L. root (1g/5ml ethanol); Column: Whatman Partisphere-5 NH_2, 25 cm x 4.6 mm (I.D.).

Table I. Analysis of ethanolic extract of dry powder of Curcuma longa L. root on PartiSphere-5 NH_2 column (Figure 1).

Peak number	Component	Percentage in the extract
1	Unknown	16.9 %
2	Bisdemethoxycurcumin	13.8 %
3	Demethoxycurcumin	13.0 %
4	Curcumin	54.5 %

root on PartiSphere-5 NH_2 column. The curcuminoids were completely resolved on PartiSphere-5 NH_2 column and coeluted on PartiSphere-5 WCX system. It has been reported that the 1,3-diketone groups of the curcumin system interact with the active sites on the silica surface (1). The possibility of hydroxyl groups on the same skeleton interacting with the surface of the stationary phase to provide a normal-phase separation can not be ruled out.

Figure 2 shows the analysis of ethanolic extract of dry powder of Curcuma longa L. root exposed to sunlight for 120 hrs on PartiSphere-5 WCX column. When analyzed on PartiSphere-5 NH_2 column, curcuminoids were absent in the sample which showed the evidence of their complete photochemical oxidation. The degradation products such as p-hydroxybenzaldehyde, vanillin, ferulic aldehyde, p-hydroxybenzoic acid, vanillic acid and ferulic acid were detected (Fig. 2). Similar results were obtained when a methanolic extract of dry

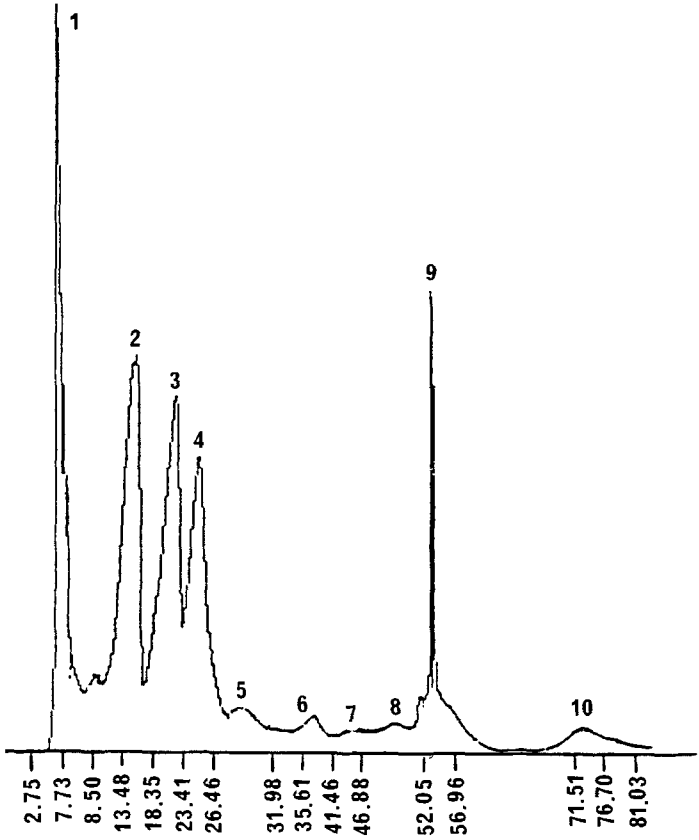

Fig. 2. 1(unknown, 22%), 2(internal standard, o-nitroaniline), 3(vanillin, 34%), 4(p-hydroxybenzaldehyde, 29%), 5(ferulic aldehyde, 0.5%), 6(p-hydroxybenzoic acid, 0.5%), 7(vanillic acid, 0.5%), 8(ferulic acid, 0.5%), 9(unknown, 8.5%) and 10(unknown, 2.5%). Mobile phase: hexane:ethanol:water (300:30:0.75, v/v); Flow rate: 0.4 ml/min.; λ max: 280nm; Sample volume injected: 50 µl + 5 ul internal standard (10mg/5ml ethanol); Sample description: ethanolic extract of dry powder of Curcuma longa L. root (1g/5ml). The sample was exposed to sunlight for 100 hrs. Column: Whatman Partisphere-5 WCX, 11 cm x 4.6 mm (I.D.) (three columns were connected in series).

Table 2. Analysis of methanolic extract of dry powder of Curcuma longa L. root on PartiSphere-5 WCX column. The extract was exposed to sunlight for 120 hrs.

Component	Percentage in the extract
Vanillin	2.0 %
p-Hydroxybenzaldehyde	2.0 %
Ferulic aldehyde	0.2 %
p-Hydroxybenzoic acid	0.2 %
Vanillic acid	1.5 %
Ferulic acid	0.1 %

powder of Curcuma longa L. root was exposed to sunlight for 120 hours and analyzed on PartiSphere-5 NH_2 and PartiSphere-5 WCX columns (Table 2).

Tables 3 and 4 represent the result of the analysis of dry powder of Curcuma longa L. root which was exposed to sunlight for 120 hours on PartiSphere-5 NH_2 and PartiSphere-5 WCX columns. The degradation products such as vanillin and p-hydroxybenzaldehyde along with curcuminoids were present.

Comparison of the Tables 1 and 3 show that the order of stability of the curcuminoids to photochemical oxidation was as follows: demethoxycurcumin > bis-demethoxycurcumin > curcumin. The curcuminoids were found to be more stable to photooxidation in dry powder of Curcuma longa L. root than in its ethanolic or methanolic extract.

Table 3. Analysis of dry powder of Curcuma longa L. root on PartiSphere-5 NH_2 column. The sample was exposed to sunlight for 120 hrs and extracted with ethanol.

Component	Percentage in the extract
Bisdemethoxycurcumin	13.9 %
Demethoxycurcumin	12.3 %
Curcumin	41.0 %

Table 4. Analysis of dry powder of Curcuma longa L. root on PartiSphere-5 WCX column. The sample was exposed to sunlight for 120 hrs and extracted with ethanol.

Component	Percentage in the extract
Bisdemethoxycurcumin, Demethoxycurcumin and Curcumin	85.0 %
Vanillin	2.0 %
p-Hydroxybenzaldehyde	2.0 %

The amounts of decomposition products produced in the various samples was in the following order: ethanolic extract > methanolic extract > dry powder. It appears that the curcuminoids undergo transformation into intermediate which further decompose to produce degradation products such as vanillin, p-hydroxybenzaldehyde and ferulic aldehyde etc.

The elution order of the curcuminoids on Whatman PartiSphere-5 NH_2 column was as follows: bisdemethoxycurcumin > demethoxycurcumin > curcumin which is similar to as reported by Tonnesen and Karlsen (5) on Nucleosil-NH_2. The degradation components were eluted in the following order: vanillin > p-hydroxybenzaldehyde >ferulic aldehyde > p-hydroxybenzoic acid > vanillic acid > ferulic acid.

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REFERENCES

1. Amakawa, E., Hirata, K., Ogiwara, T. and Phnishi, K., Jpn Anal., 23, 586 (1981).

2. Khurana, A. L., Butts, E. T. and Ho, C.-T., J. Liq. Chromtogr, (in press).

3. Kolstad, B., Nat., 167 (1980).

4. Krishnamurthy, M. N., J. Food Sci. Technol., 12, 12 (1980).

5. Tonnesen, H. H. and Karlsen, J., Lebensm. Unters. Forsch., 177, 348 (1983).

6. Tonnesen, H. H. and Karlsen, J. Lebensms. Unters. Forsch., 180, 132 (1985).

7. Tonnesen, H. H., Karlsen, J. and Henegouwen, G. B., Lebensm, Unters. Forsch., 180, 116 (1986).