ELSEVIER

Journal of Food Engineering 56 (2003) 257–259

JOURNAL OF FOOD ENGINEERING

www.elsevier.com/locate/jfoodeng

Studies on the stability of turmeric constituents

Erika Pfeiffer [a], Simone Höhle [a], Aniko M. Solyom [b], Manfred Metzler [a,*]

[a] *Institute of Food Chemistry and Toxicology, University of Karlsruhe, P.O. Box 6980, 76128 Karlsruhe, Germany*
[b] *Arizona Center for Phytomedicine Research, University of Arizona, P.O. Box 210207, Tucson, AZ 85721-0207, USA*

Received 21 October 2001

Abstract

In order to investigate the stability of curcuminoids in physiological media, two samples with different composition of curcumin (CUR I), mono-demethoxycurcumin (CUR II) and bis-demethoxycurcumin (CUR III) were incubated in phosphate buffer and cell culture medium without or with fetal calf serum. The curcuminoids decomposed very rapidly (more than 90% within 12 h) when serum was omitted, but were more stable in the presence of serum. The stability differed between the curcuminoids: CUR I was the least, and CUR III was the most stable curcuminoid. Several degradation products of CUR I were detected, most of which were not yet identified; ferulic acid and vanillin were disclosed as minor products.

Keywords: Curcuminoids; Stability

1. Introduction

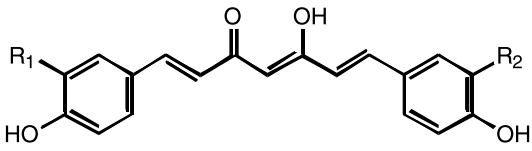
The powdered dry rhizome of the plant *Curcuma longa*, commonly called turmeric, is widely used as a coloring agent and spice in many food items. In several Asian countries, it has also been used for centuries as a traditional remedy for the treatment of inflammation and other diseases (Ammon & Wahl, 1991). The yellow pigment of turmeric, which is composed of curcumin (CUR I), mono-demethoxycurcumin (CUR II) and bis-demethoxycurcumin (CUR III) has been reported to possess anti-oxidative, anti-inflammatory and anti-carcinogenic properties (Azuine, Kayal, & Bhide, 1992). Since these curcuminoids have a diphenolic structure (Fig. 1) similar to that of known phytoestrogens, we intend to study the estrogenicity of curcuminoids in cultured mammalian cells. Because the in vitro assays for estrogenicity require longterm incubation of the cells with the test compound, we have first studied the stability of commercial curcumin (cCUR) and a fraction of turmeric extract (CUR F4) in aqueous media.

2. Methods

The samples of cCUR and CUR F4, which had a different composition of CUR I, II, and III according to HPLC analysis (Fig. 1), were dissolved in DMSO and added to 0.1 M phosphate buffer pH 7.4 or culture medium without or with 5% or 10% fetal calf serum (FCS) (final DMSO concentration 1%). Various concentrations of curcuminoids ranging from 5 to 100 μg/ml were used. Incubations without Ishikawa cells were carried out at 37 °C for various time periods. For incubations with cells, 96-well microtiter plates containing 20,000 cells per well were kept at 37 °C for 48 h in a 5% CO_2 atmosphere. The number of viable cells was then determined using an electronic cell counter and compared to untreated controls in order to measure cytotoxicity. For the analysis of the incubation mixtures, aliquots of 0.5 ml were extracted with ethylacetate and separated by HPLC on a reversed phase Prodigy 5ODS(2) column (250 × 4.6 mm; 5 μm) with a linear gradient of acetonitrile in water (from 20% to 70% acetonitrile in 30 min). The flow rate was 1 ml/min and diode array detection was used.

* Corresponding author. Tel.: +49-721-608-2132; fax: +49-721-608-7255.
E-mail address: manfred.metzler@chemie.uni-karlsruhe.de (M. Metzler).

0260-8774/02/$ - see front matter
PII: S0260-8774(02)00264-9



		cCUR	CUR F4
CUR I	$R_1 = OCH_3$; $R_2 = OCH_3$	71.5%	38.7%
CUR II	$R_1 = OCH_3$; $R_2 = H$	19.4%	25.7%
CUR III	$R_1 = H$; $R_2 = H$	9.1%	35.6%

Fig. 1. Chemical structures and composition of the curcuminoids.

3. Results and discussion

The solubility of both samples depends on the serum content of the medium: precipitation started at 20 μg/ml in medium without FCS, at 50 μg/ml with 5% FCS, and at 100 μg/ml with 10% FCS. In incubation with 5% FCS, 5 μg/ml cCUR was slightly toxic (83% viable cells), whereas 10 μg/ml exhibited pronounced cytotoxic effects (15% viability).

When the effect of concentration and time on the stability of the curcuminoids was studied (Fig. 2), we observed that all three curcuminoids were unstable. Stability was markedly lower in the absence of serum in the medium. Furthermore, the three curcuminoids displayed different stabilities: CUR I was the least and CUR III the most stable curcuminoid. The instability was independent of the composition, but depended on the concentration of the curcuminoids and was most pronounced at low concentrations, which were non-cytotoxic for Ishikawa cells.

HPLC analysis of the incubation mixture of cCUR in phosphate buffer (Fig. 3A) revealed several as yet unidentified decomposition products (peaks 1, 4, 5), which were also found when pure CUR I was incubated (Fig. 3B). No decomposition products were detected in incubations of pure CUR II and III (data not shown). Incubation of cCUR with Ishikawa cells gave rise to six additional products (Fig. 3C, peaks 2, 3, 6, 7, 8, 9), two of which were identified by their UV spectra and cochromatography with authentic reference compounds as

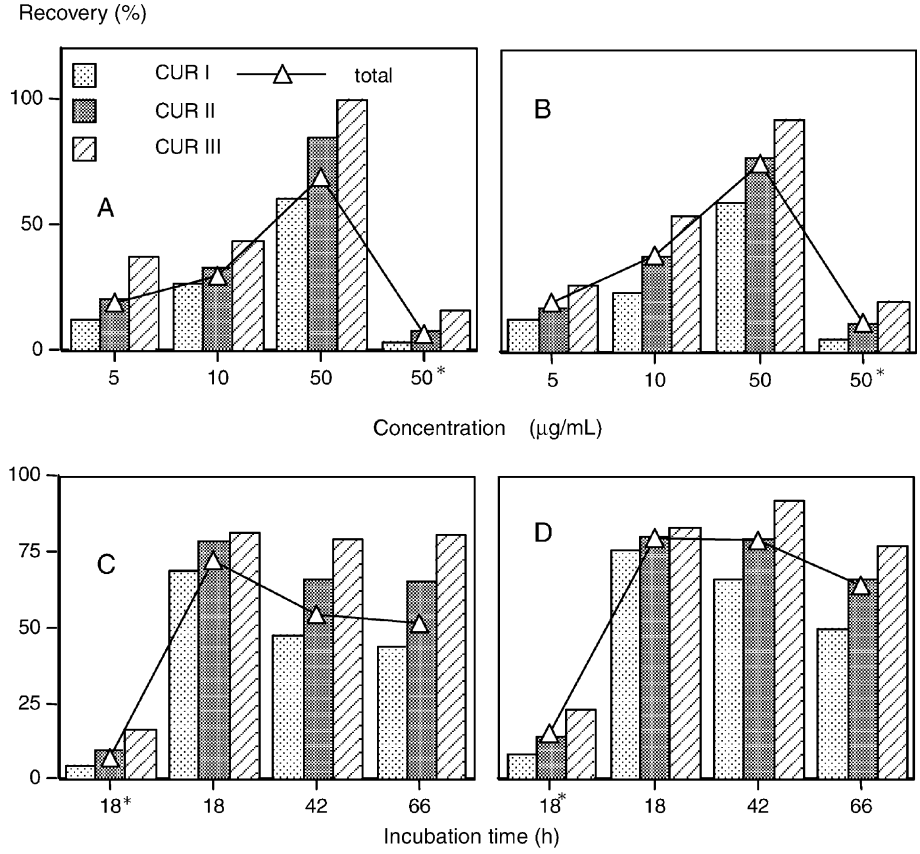


Fig. 2. Recovery of the curcuminoids after 18 h incubation at various concentrations of cCUR and CUR F4 in medium with 5% FCS and cells (A, B) and 100 μg/ml cCUR and CUR F4 in medium with 10% FCS and cells at different incubation times (C, D). * Incubation in medium without FCS and cells.

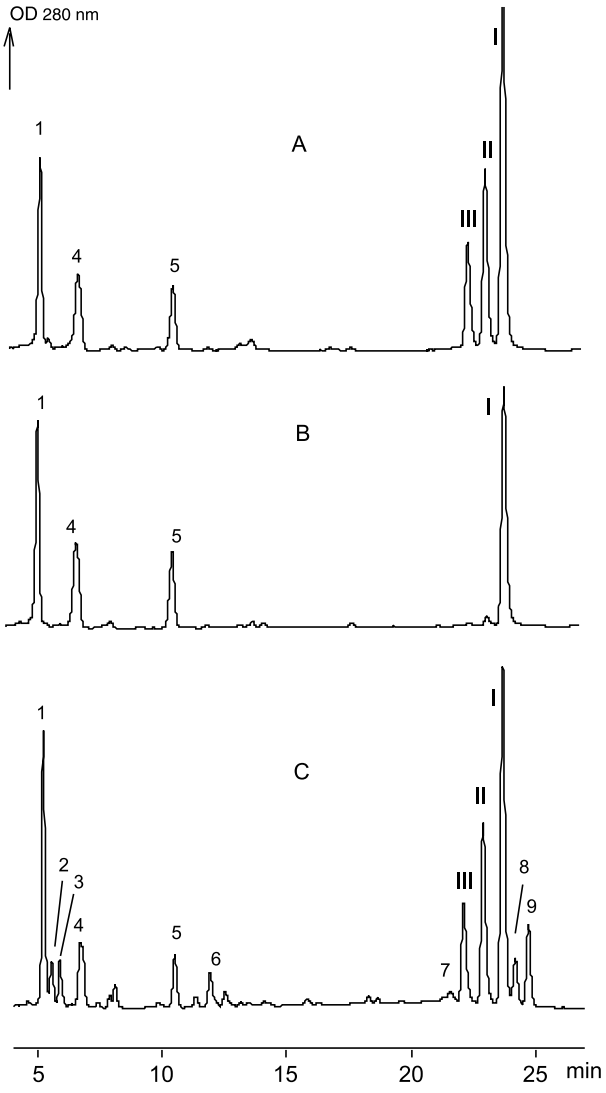


Fig. 3. HPLC profiles of the incubation of (A) cCUR (phosphate buffer, 2 h, decomposition 52.6%), (B) pure CUR I (phosphate buffer, 2 h, decomposition 73.2%), and (C) cCUR (medium plus FCS plus cells, 18 h, decomposition 72.8%). Concentration was 5 μg/ml in all cases.

ferulic acid (peak 2) and vanillin (peak 3); peak 6 is yet unknown and peaks 7, 8 and 9 were tentatively identified as curcuminoids with one reduced double bond according to their UV spectra, which represent possible metabolites. The instability of CUR I, but not CUR II and III, has already been reported by Wang et al. (1997). Vanillin, ferulic acid, feruloylmethane and *trans*-6-(4-hydroxy-3-methoxyphenyl)-2,4-dioxo-5-hexenal were identified as degradation products. Further studies are needed to elucidate the chemical structures and cytotoxic effects of all degradation products and metabolites.

In summary, the different stability of the curcuminoids and formation of decomposition products and putative metabolites will have to be taken into account when the estrogenicity or other effects of these bioactive compounds are determined.

Acknowledgements

Supported by NIH Grant P50 AT00474 to the Arizona Center for Phytomedicine Research.

References

Ammon, H. P., & Wahl, M. A. (1991). Pharmacology of *Curcuma longa*. *Planta Medica, 57*(1), 1–7.

Azuine, M. A., Kayal, J. J., & Bhide, S. V. (1992). Protective role of aqueous turmeric extract against mutagenicity of direct-acting carcinogens as well as benzo [alpha] pyrene-induced genotoxicity and carcinogenicity. *Journal of Cancer Research and Clinical Oncology, 118*(6), 447–452.

Wang, Y. J., Pan, M. H., Cheng, A. L., Lin, L. I., Ho, Y. S., Hsieh, C. Y., & Lin, J. K. (1997). Stability of curcumin in buffer solutions and characterization of its degradation products. *Journal of Pharmaceutical and Biomedical Analysis, 15*(12), 1867–1876.