HPLC Analysis of Curcuminoids in Dietary Supplements and in vitro Digestion Fractions

[1]N.E.Craft, [2]C.Chitchumroonchokchai, [2]M.L.Failla and [3]H.N.Bhagavan

[1]Craft Technologies, Inc., Wilson, NC, [2]Dept of Human Nutrition, Ohio State University, Columbus, OH and [3]Tishcon Corp., Westbury, NY

www.crafttechnologies.com

BACKGROUND

Curcumin (Cur), demethoxycurcumin (DMC), and bis-demethoxycurcumin (BDMC) are the main curcuminoids in Curcuma longa used to prepare the spice turmeric. Cur has been shown to reduce the risk of cancer, inflammation and Alzheimers. As such, it has become increasingly popular in foods and dietary supplements. To examine content and bioaccessibility of commercial Cur products, we developed and validated an HPLC method to measure Cur and metabolites. Dietary supplements were processed via an in vitro digestion (simulated gastric and small intestinal phases) and Caco-2 cellular uptake (Chitchumroonchokchai et al., J. Nutr. 2004). Cur, DMC, BDMC were monitored at 425nm for sensitive detection and 280nm was monitored to detect the internal standard, Flavone, and cellular metabolites of curcuminoids. Three products were tested containing from 10% to 95% curcuminoids.

OBJECTIVES

To assess relative bioavailability of 2 commercial Curcumin products using an simulated digestion coupled to cellular uptake by human intestinal cells (Caco-2 cells) to assess Cur micellarization and cellular uptake. The percentage of Cur, DMC, and BDMC in the fractions was measured using HPLC with UV and visible detection.

METHODS

The Curcumin products were provided to Craft Technologies, Inc. and the Dept. of Human Nutrition, Ohio State University by Tishcon Corp. The amounts of Cur DMC, and BDMC in products were determined by HPLC at Craft Tech. At OSU samples were subjected to simulated gastric and small intestinal digestion. The filtered aqueous fraction of small intestinal digestate was diluted with cell culture medium and added to differentiated cultures of Caco-2 human intestinal cells to assess cellular accumulation. Aliquots of digestate, filtered aqueous fractions, and cell pellets were shipped frozen to Craft Tech. for analysis of curcuminoids. Samples of cells, digesta, apical and basolateral media were hydrolyzed with glucuronidase/sulfatase from Helix pomatia and mixed with the internal standard, Flavone. The samples were extracted twice with a mix of ethyl acetate and methanol. The combined organic extract was dried in a SpeedVac then redissolved in mobile phase.

HPLC Conditions:

Column: Hyperclone C18, 3um, 4.6 x 150 mm
Mobile phase: Acetonitrile/methanol/water/acetic acid (36:8:54:2)
Flow rate: 1.0 mL/min
Detection: 425nm and 280nm
Internal Std: Flavone (~4 mcg/mL final)
Calibration: 0.2 to 20 mcg/mL
Approximate retention times: Flavone, 9.5 min; BDMC, 11 min; DMC,12.5 min; Cur, 14 min

Figure 1

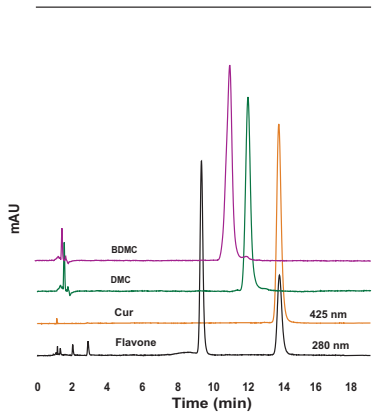


Figure 1. Chromatogram Overlay of Curcuminoid Standards at 425nm (upper three traces) and Cur with the Internal Standard, Flavone, at 280nm (lower trace).

Figure 2

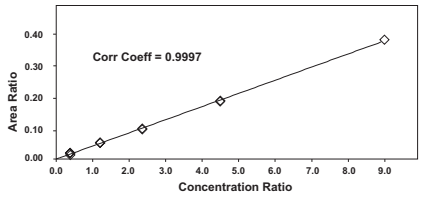


Table 1

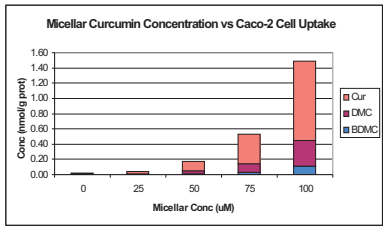
Product Descriptions

	BDMC	DMC	CUR	Total
Softgel	13.1 (2.9%)	74.4 (16.5)	366 (80.6%)	454
Powder	9.5 (10.6%)	20.1 (22.5%)	59.7 (66.8%)	89.4

Values are expressed as mg/g. Values in parentheses are % of Total Curcuminoids
Tishcon BCM 95 Softgels, Batch # 1171-6090 with average fill wt. of 0.513g (Softgel), and Naturex Turm'Brite 10% sd Powder, Batch # 159/09/A6 (Powder)

Figure 3

A



B

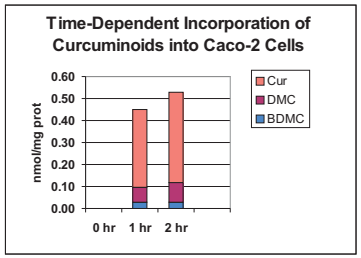


Figure **3**. Cell accumulation of Curcuminoids from synthetic micelles is proportional to medium concentration (**A**) and to length of exposure (**B**). **A**. Cultures were exposed to medium with indicated concentrations of Total Curcuminoids for 4 h. **B**. Cells were incubated in medium containing 50 μmol/L Curcuminoids for 0, 1, 2 h.

ACKNOWLEDGMENTS

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Figure 4

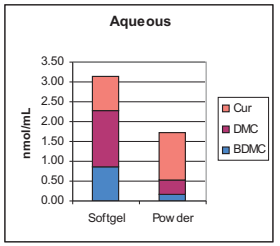

Figure 4. The efficiency of micellarization of Curcuminoids in softgel and powder supplements after simulated in-vitro digestion. Curcuminoids were extracted from the micellarized fractions and analyzed by HPLC.

Figure 5

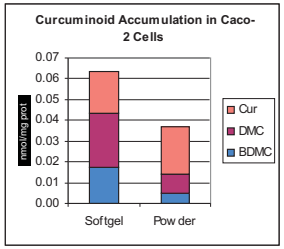

Figure 5. Uptake of micellarized Curcuminoids by Caco-2. Monolayers of Caco-2 (HTB 37 P27, 11dpc) were incubated for 4 h with serum free DMEM containing micelles generated during simulated digestion.

RESULTS

Figure 1. Separation of curcuminoids and incorporation of Flavone as an internal standard.

Figure 2 is a representative calibration curve of Cur.

Figure 3 illustrates that Caco-2 cells transported the Curcumin from the micelles in a concentration- (A) and time-dependent (B) manner.

Figure 4 illustrates the pmol Curcuminoids taken up per mg protein.

Figure 5 illustrates that cellular uptake is associated with the amount of Curcumin that is incorporated into the micellar fraction during the simulated digestion.

The softgel resulted in greater % micellarization and higher uptake by the cells. There was evidence that curcuminoids were further metabolized or transported across the basolateral surface.