JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Curcumin Bioavailability from Enriched Bread: The Effect of Microencapsulated Ingredients

Paola Vitaglione,[†] Roberta Barone Lumaga,[†] Rosalia Ferracane,[†] Irena Radetsky,[‡] Ilario Mennella,[†] Rita Schettino,[†] Saul Koder,[§] Eyal Shimoni,[‡] and Vincenzo Fogliano*[,†]

[†]Department of Food Science, University of Naples, via Università 100, 80055 Portici (NA), Italy

[‡]Technion Israel Inst Technol, Fac Biotechnol & Food Engn, IL-32000 Haifa, Israel

[§]Karmat Coating Industries Ltd., M.P. Hevel Megido, 19245 Kibbutz Ramot Menashe, Israel

ABSTRACT: Human bioavailability of curcumin from breads enriched with 1 g/portion of free curcumin (FCB), encapsulated curcumin (ECB), or encapsulated curcumin plus other polyphenols (ECBB) was evaluated. Parental and metabolized curcuminoids and phenolic acids were quantified by HPLC/MS/MS in blood, urine, and feces collected over 24 h. The concentrations of serum curcuminoids were always below 4 nmol/L and those of glucuronides 10-fold less. Encapsulation delayed and increased curcuminoid absorption as compared to the free ingredient. Serum and urinary concentrations of ferulic and vanillic acid were between 2- and 1000-fold higher than those of curcuminoids, with ECBB eliciting the highest amounts. Fecal curcuminoids were 6-fold more abundant after ECB than FCB, while phenolic acids after ECBB quadruplicated those after ECB. Curcuminoid encapsulation increased their bioavailability from enriched bread, probably preventing their biotransformation, with combined compounds slightly reducing this effect. Phenolic acids are the major metabolites of curcuminoids and may contribute to their biological properties.

KEYWORDS: *bioavailability, curcumin, encapsulation, functional food*

INTRODUCTION

Curcumin is commonly used in food products, mainly as coloring agent. Several biological properties have been attributed to this compound mainly related to its ability to inhibit NF-kB activation.[1] Curcumin has been proposed as a potential therapeutic agent against several non communicable chronic diseases having an inflammatory origin such as neurodegenerative diseases (Alzheimer's and Parkinson's disease, multiple sclerosis, epilepsy), cardiovascular diseases (CVD), diabetes, obesity, allergies, and certain types of cancer.[2]

Although clinical studies in humans proved that curcumin is safe and well tolerated even at very high doses (8−12 g/die), its use as a therapeutic agent is limited by its low bioavailability, poor absorption, rapid metabolism, and systemic clearance.[3,4]

Drug delivery systems such as nanoparticles, liposomes, microemulsions, and polymeric implantable devices are emerging as viable alternatives that can be used to deliver therapeutic concentrations of various chemopreventive agents such as curcumin, ellagic acid, green tea polyphenols, and resveratrol into the systemic circulation.[5]

Several absorption enhancers have also been used to improve curcumin bioavailability. Piperine enhanced the bioavailability both in preclinical studies and in studies on human volunteers.[6] This was attributed to the ability of piperine in reducing first-pass metabolism.[6] Animal studies also demonstrated that inclusion of curcumin into nanoparticles caused at least a 9-fold increase in oral bioavailability when compared to curcumin administered alone or with piperine.[7] On the other hand, interactions among bioactive compounds that may positively influence oral bioavailability of individual molecules are known for genistein toward epigallocatechingallate[8] as well as for several natural bioactive compounds (quercetin, hesperitin, curcumin, piperin, and naringenin) with P-glycoprotein-inhibiting activity, toward some anticancer drugs.[9,10]

Encapsulation may confer new properties and potentials to bioactive compounds through modification of physical and nutritional properties.[5] This may be of particular interest in the formulation of functional foods, where technological and nutritional aspects must be strictly considered.[11] In this respect, selecting suitable coating materials can increase water solubility of bioactive compounds and permit their controlled delivery into the gastrointestinal tract.[5]

In this framework, the aim of this study was to evaluate the bioavailability of curcumin from different types of bread containing curcumin in different forms: free and microencapsulated in a cellulose derivative coating containing curcumin alone or in combination with a mixture of three bioactive compounds including piperine, quercetin, and genistein. A crossover, randomized, single blind study in healthy subjects was performed. Curcuminoid bioavailability over 24 h following consumption of the breads was assessed by HPLC/MS/MS determining blood, urine, and fecal concentrations of curcuminoids, their metabolites (glucuronides, sulphated, and reduced compounds), and several phenolic acids.

Received: November 5, 2011
Revised: January 29, 2012
Accepted: March 8, 2012
Published: March 8, 2012

Table 1. Composition of the Functional Ingredients Included in the Different Types of Bread

	core				coating		
	curcuminoids	piperine	quercetin	genistein	cellulose derivative (Ethocell 100)	castor oil	HVO
FC (free curcumin)	95%						
EC (encapsulated curcumin)	72.68%				7.48%	1.02%	15%
EC+B (encapsulated curcumin + other polyphenols)	66.5%	1.0%	1.0%	1.0%	6.84%	0.93%	13.73%

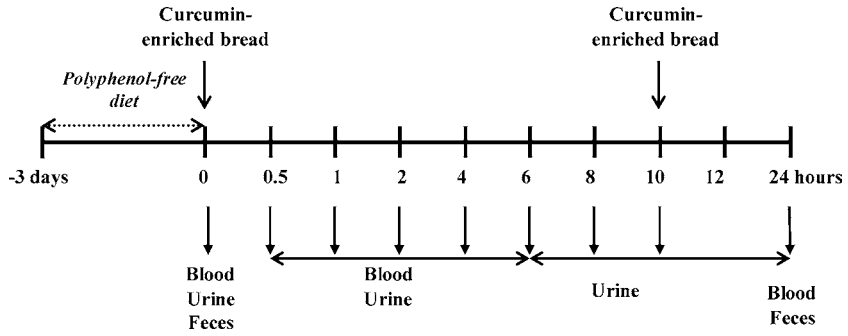

Figure 1. Study design. Each subject followed this time schedule for each type of curcumin-enriched bread by a crossover double-blind randomized design.

MATERIALS AND METHODS

Chemicals. All chemicals and reagents were of analytical grade. Methanol, water, and acetonitrile were from Merck (Darmstadt, Germany); ethyl acetate, glacial acetic acid, and hydrochloric acid were from Clean Consult International (Lodi, Italy); formic acid (98% purity) and butylated hydroxytoluene (≥99%) were obtained from Sigma (St. Louis, MO). All analytical standards chlorogenic acid (95%), ferulic acid (99%), 4-hydroxyphenylacetic acid (HPA, 98%), 3-(4-hydroxyphenyl)propionic acid (HPP, 98%), vanillic acid (97%), and curcumin (≥80%) were purchased from Sigma (St. Louis, MO).

Curcumin Ingredients and Breads. Three types of curcumin containing ingredients were used, free curcumin (FC), encapsulated curcumin (EC), and encapsulated curcumin, plus three bioactive compounds, piperine, quercetin, and genistein (EC+B).

FC was a 95% pure curcuminoid extract from turmeric and was constituted by 79% curcumin, 19% desmethoxycurcumin, and 2% bisdesmethoxycurcumin. EC and EC+B were obtained by fluidized bed spray coating, followed by bottom spray. Curcumin was encapsulated by double coating, whereas the inner coating material of microcapsules was constituted by cellulose derivative (Ethocel 100, Dow Chemicals) as a first layer, and hydrogenated vegetable oil (HVO) as an external layer. Ethocel 100 (88% Ethocel 100 and 12% liquid castor oil, as emulsifier) was dissolved in 80% acetone and 20% methanol to get a 4% w/w solution for coating, and hydrogenated vegetable oil was melted by heating to 95 °C prior to coating. The particles of curcumin were placed at the bottom of the chamber and blown upward by hot air. The coating polymer solution (Ethocel 100) and HVO were sprayed upward in the same direction, one by one. In this way, curcumin particles pass through a simultaneous coating (drying) environment upward by reaching the top of the chamber; the partially coated particles move downward and undergo further drying until the desired coat thickness is reached.

Finally, EC and EC+B contained 72.7% and 66.5% of curcuminoids, respectively; in EC+B, piperine, quercetin, and genistein, 1.0% of each, were also present (Table 1). All combined compounds were encapsulated one by one with a single layer of polymer solution (Ethocel 100) by different amounts of coatings (0−20% coat), mixed together to achieve controlled release mechanism. Final moisture, for both EC and EC+B, was <5%, and the coating/drying process ensured a total removal of organic solvents derived from the polymer solution.

Each ingredient was included in a classical bread recipe, and three types of bread containing 1 g of curcuminoids in a 100 g portion were formulated. Total phenolic acids in these breads were measured. They were at the same amount in all types of bread, and in particular total phenolic acids were 3.7 ± 0.5 mg/100 g of which ferulic acid was 2.2 ± 0.2 mg/100 g, vanillic acid was 1.4 ± 0.5 mg/100 g, and cumaric acid was 0.1 ± 0.05 mg/100 g. In addition, the bread with EC+B also contained 0.01 g of piperine, 0.01 g of quercetin, and 0.01 g of genistein. Depending on the ingredient used, the breads will be hereinafter indicated as: FC bread (FCB), EC bread (ECB), and EC+B bread (ECBB). They were produced in laboratory scale, and curcumin bioavailability upon their consumption was studied. By consuming one portion of bread, subjects ingested 0.8 g (2.1 mmol) of curcumin, 0.2 g (591.7 μmol) of desmethoxycurcumin, and 0.08 g (259.7 μmol) of bisdemethoxycurcumin (3.0 mmol of total curcuminoids).

Subjects and Treatment. The protocol of the study was approved by the Ethics Committee of "Federico II" University of Naples (Approval Number: 37/10).

Ten healthy subjects, age 31 ± 2 years, BMI of 23.5 ± 1.2 kg/m^2, were enrolled. Subjects with gastrointestinal pathologies and/or metabolic disease, those taking anti-inflammatory drugs, or those under controlled diet in the previous 6 months were excluded from the study. Volunteers signed a written informed consent before starting the experimental protocol, consisting of a double-blind randomized crossover study as schematized in Figure 1. Volunteers were asked to follow a polyphenol-free diet for 3 days before and over the experiment days. Thus, they were recommended to exclude from their diet all polyphenol-rich foods and beverages such as fruits, vegetables, chocolate, tea, coffee, wine, beer, supplements, herbal extracts, and whole grains-based foods. Assumption of nonsteroidal anti-inflammatory drugs (NSAID) was also avoided during 1 week before the study. On the experiment day, at 8:00 a.m., 12 h-fasted subjects reached the laboratory and were randomized (using a computer generated random sequence) to receive, in a blinding manner, one of the three types of experimental bread. A different codification of breads by an external researcher allowed the double blindness of the study until the final data analysis. One portion of the bread was served to each participant, and it was entirely consumed at the moment (for breakfast) within 15 min. Before consumption of bread and after 30 min, 1 h, 2 h, 4 h, and 6 h, blood drawings were performed. Urine volume was measured over 24 h, and 10 mL samples were collected before and at 0−2 h, 2−4 h, 4−6 h, 6−8 h, 8−10 h, and 10−24 h time intervals postbread ingestion. After 6 h from breakfast ingestion, subjects were given another portion of the same bread type they had consumed in the morning, then they left the research center and consumed their lunch (always choosing among allowed foods). The second bread portion

Table 2. Protonated Molecules and Product Ions of Compounds Analyzed by HPLC/MS/MS and MS Parameters

		product ions					CE			CXP			
compound	M−H	1	2	3	DP	FP	1	2	3	1	2	LOD (ng/mL)	LOQ (ng/mL)
curcumin	367.1	217.1	148.9		−46	−400	−16	−25		−10		0.2	0.5
desmethoxycurcumin	337	217			−38	−400	−17			−10		0.5	1.0
bisdesmethoxycurcumin[a]	307	217			−46	−400	−49			−6			
curcumin glucuronide[a]	543.1	367			−60	−375	−30			−7			
curcumin sulfate[a]	447	367			−60	−375	−25			−7			
tetrahydrocurcumin glucuronide[a]	547	135			−60	−375	−25			−7			
hexahydrocurcumin[a]	373	179			−60	−375	−25			−7			
hexahydrocurcumin glucuronide[a]	549	373			−60	−375	−30			−7			
vanillic acid	167	152	108	123	−45	−250	−22	−26		−9	−11	2.5	5.0
ferulic acid	192.8	133.9	177.9		−35	−250	−22	−17		−10		0.5	1.0
chlorogenic acid	353	191			−35	−250	−21			−8		0.5	1.0
3,4-dihydroxyphenylacetic acid (diHPA)	167	123.1			−30	−250	−11			−7		0.5	1.0
4-hydroxyphenylacetic acid (HPA)	151	107	78.9		−35	−250	−16	−25		−7		3.0	5.0
3-(4-hydroxyphenyl)propionic acid (HPP)	164.9	121	105.9	76.7	−25	−250	−10	−20	−10	−7		25.0	100.0

[a]It was identified based on molecular weight and specific fragmentation patterns as reported in ref 12; the quantification was obtained using the calibration curve of curcumin.

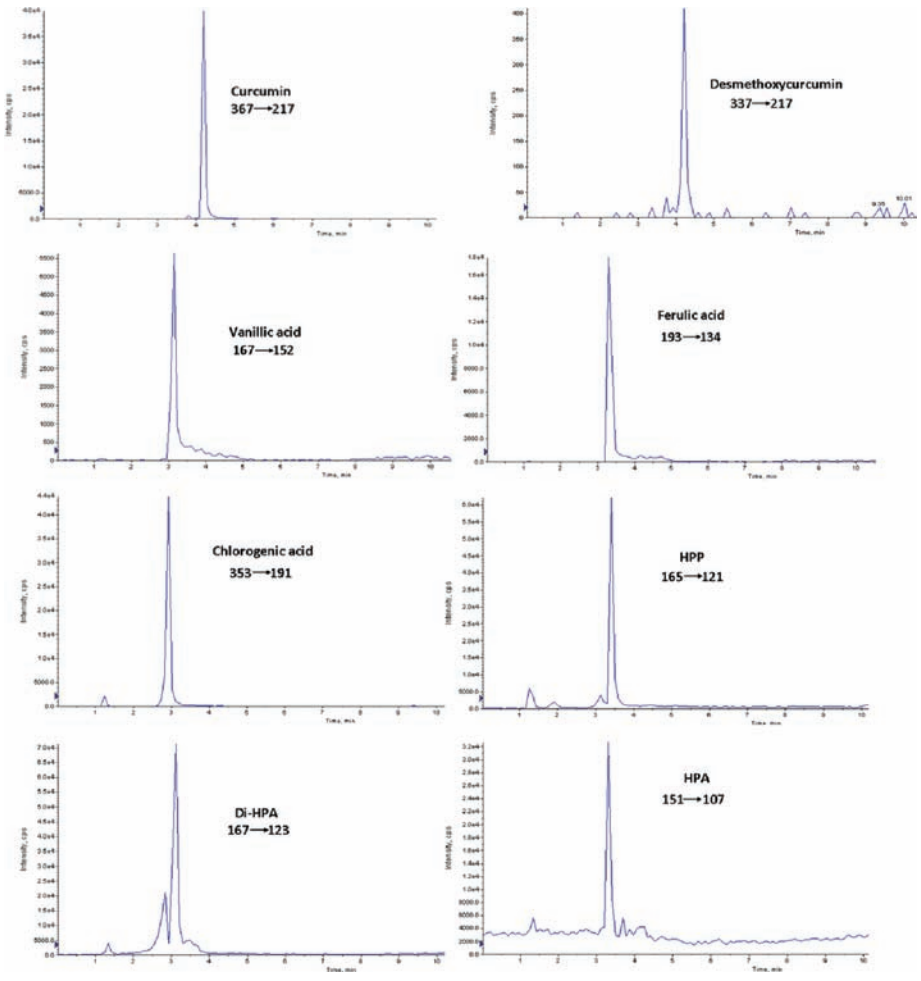

Figure 2. Typical MRM chromatograms obtained by HPLC/MS/MS analysis on urine of subjects consuming ECBB. The monitored transitions relative to the main compounds retrieved in the samples are indicated beside each panel.

was consumed at dinner (within 10:00 p.m.), and subjects were asked to weigh and record any amount of the bread that they failed to consume. The day after the experiment, 12 h-fasted participants returned to the laboratory; they were submitted to a blood drawing (24 h from the first bread consumption) and left the faecal sample collected on the experiment day.

dx.doi.org/10.1021/jf204517k | J. Agric. Food Chem. 2012, 60, 3357−3366

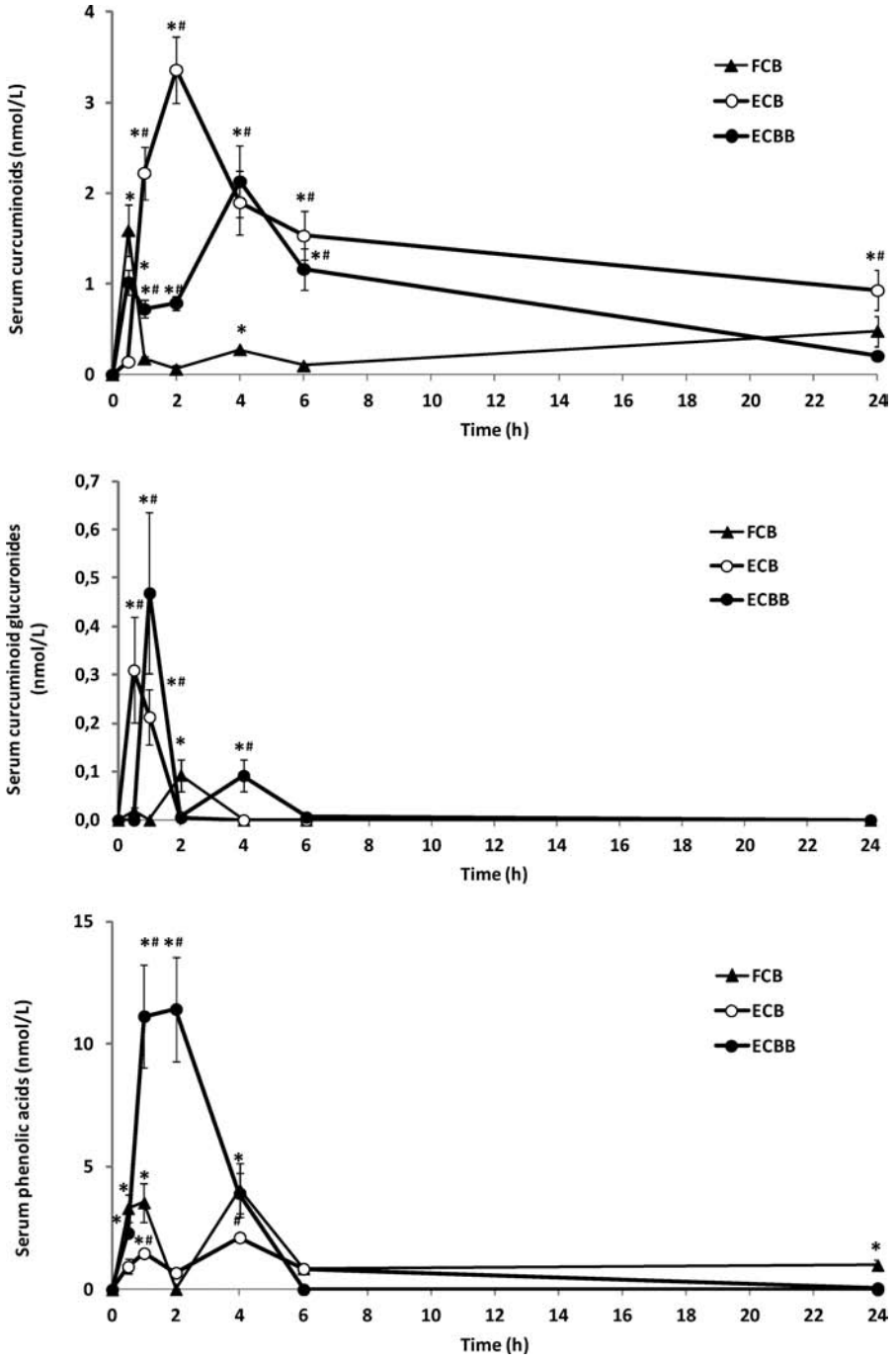

Figure 3. Serum concentration−time curves of total curcuminoids, curcuminoid glucuronides, and phenolic acids following consumption of FCB, ECB, and ECBB. *p < 0.05 versus baseline; #p < 0.05 versus FCB.

After a 1 week wash-out period during which subjects returned to consume their own habitual diet, participants were asked again to switch to the polyphenol-free diet, and after 3 days they were crossed-over to receive a new treatment with a different experimental bread. Following this experimental design, all subjects completed the study receiving all three treatments, including two daily bread portions for each. All subjects were successfully compliant with each treatment.

Biological Sample Treatment. Blood samples were collected in Vacutainer tubes for gel separation, and immediately centrifuged at 4000 rpm for 10 min at 4 °C. Urine samples were immediately treated with 0.005% (w/v) of butylated hydroxytoluene (BHT). Feces were diluted 1:10 (W/V) in PBS 10 mM, containing 0.005% of BHT, vortexed, and centrifuged at 4000 rpm for 15 min at 4 °C. Serum, urine, and fecal supernatants were stored at −40 °C until the analysis.

One milliliter of serum and 3 mL of urine and fecal samples were extracted, by 6 and 9 mL of ethyl acetate, respectively. The supernatants were dried under nitrogen flow and dissolved in 50 μL of methanol/water (70:30). Each sample was extracted in duplicated. Twenty microliters was injected in the HPLC/MS/MS system.

HPLC/MS/MS Analysis. Chromatographic separation of curcumin and metabolites was performed with a HPLC system consisting of 2 micropumps by Perkin-Elmer (U.S.) Series 200. Elution was achieved with a Phenomenex Luna 3 μ C18(2) 100 A (50 × 2.00 mm) column and by using the following mobile phases: A = H_2O/acetonitrile/formic acid 94.9:5:0.1 (v/v/v), and B = acetonitrile/formic acid 99.9:0.1; (v/v); the flow rate was 200 μL/min. A linear gradient was applied as follows: 0−1 min, 4−40% B; 1−3 min, 40−100% B; 3−5 min, 100% B; 6−10 min, 4% B. Analysis was performed using an API

Table 3. Serum Pharmacokinetic Data (Mean ± SEM)[a]

	C_{max} (nmol/L)			t_{max} (h)			AUC_{0-6} (nmol/L·h)		
	FCB	ECB	ECBB	FCB	ECB	ECBB	FCB	ECB	ECBB
curcuminoids									
curcumin	1.44 ± 0.53	0.28 ± 0.01	1.12 ± 0.66	0.5	1	6	1.45 ± 0.32	0.21 ± 0.01	4.08 ± 1.61
desmetoxy-curcumin	0.15 ± 0.03	0.27 ± 0.17	0.52 ± 0.15	0.5	6	0.5	0.22 ± 0.19	0.75 ± 0.42	1.38 ± 0.6
bisdesmethoxy-curcumin	n.d.	3.33 ± 1.08	1.1 ± 0.78	n.d.	2	4	n.d.	11.15 ± 4.82	2.2 ± 1.55
total curcuminoids	1.59 ± 0.28	3.36 ± 0.36	2.13 ± 0.39	0.5	2	4	1.67 ± 0.51	12.11 ± 5.25	7.66 ± 3.76
glucuronides									
curcumin-glucuronide	0.06 ± 0.04	0.13 ± 0.05	0.35 ± 0.25	2	1	1	0.1 ± 0.1	0.2 ± 0.11	0.33 ± 0.23
hexahydrocurcumin-glucuronide	n.q.	0.13 ± 0.09	0.11 ± 0.08	n.q.	0.5	1	n.q.	0.12 ± 0.09	0.22 ± 0.15
total glucuronides	0.09 ± 0.07	0.31 ± 0.22	0.47 ± 0.33	2	0.5	1	0.15 ± 0.13	0.32 ± 0.2	0.55 ± 0.38
phenolic acids									
chlorogenic acid	1.75 ± 0.58	0.47 ± 0.3	9.69 ± 6.25	0.5	0.5	1	4.25 ± 1.22	0.55 ± 0.54	16.67 ± 10.12
derulic acid	3.46 ± 1.58	0.91 ± 0.00	9.75 ± 5.52	1	1	2	9.03 ± 5.24	2.77 ± 0.00	16.51 ± 8.28
vanillic acid	n.d.	n.d.	0.87 ± 0.61	n.d.	n.d.	2	n.d.	n.d.	1.30 ± 0.92
total phenolic acids	4.06 ± 1.11	1.27 ± 0.10	11.43 ± 2.13	4	1	2	13.28 ± 6.46	3.32 ± 0.54	34.48 ± 19.32

[a]n.d., not detected; n.q., not quantifiable.

3000 Triple Quadrupole mass spectrometer (Applied Biosystem Sciex). For identification and quantification of compounds, ionization in negative mode was used, and a multiple reaction monitoring (MRM) analysis was employed tracking the transition indicative of parent and product ion specific for each compound. Previous direct infusion experiments were performed to optimize following parameters: capillary voltage, focusing potential, entrance potential, declustering potential, and collision energy. After performing infusion, the following parameters were fixed: dwell time, 100 ms; nebulizer gas, 10; curtain gas, 12; auxiliary gas temperature, 400 °C; auxiliary gas flow rate, 6000 cm^3/min; capillary voltage, −3700 V; entrance potential, −10 V. Detailed transitions for parent molecules and product ions, MS parameters (declustering potential, DP; focusing potential, FP; collision energy, CE; collision cell exit potential, CXP), and limits of detection (LOD) and quantification (LOQ) are listed in Table 2.

Bisdesmethoxycurcumin and curcumin metabolites (tetrahydrocurcumin, hexahydrocurcumin, and hexahydrocurcumin-glucuronide) were identified comparing molecular weight and fragmentation patterns with those reported in the literature.[12,13] Quantification for curcumin metabolites was obtained using curcumin calibration curves. The specificity of the analytical method was evaluated through the analysis of sample matrices (for serum, urine, and fecal suspension) before and after spiking with pure compounds when possible; no interferences from matrix components and a good resolution for all analytics were observed. The method was validated at three different concentration levels (low, medium, and high), specific for each compound (in function of LOD), and five replicates of extraction were performed for each level. Intraday and interday accuracy and precision, expressed as % CV (coefficient of variation), were, for all compounds, always <15%. A typical HPLC/MS/MS separation of compounds in urine of subject consuming ECBB detected by MRM analysis is reported in Figure 2.

Statistical Analysis. The number of subjects was based on power calculations derived from our previous study.[14] We calculated that, at $\alpha = 0.05$ with a power of 80%, 8 subjects would allow us to detect a 20% difference in serum and urinary concentrations of parental compounds, glucuronides, and phenolic acids.

The results from HPLC/MS/MS analysis of curcumin and metabolites were analyzed and reported as the absolute changes from the baseline to reduce possible effects of intersubject fasting variability. All data were expressed as means ± SEM. The area under the curve (AUC) for each compound from baseline over 6 h after first bread portion consumption in the case of serum samples and over 0−10 h and 10−24 h for urine samples were estimated using the linear trapezoidal rule. Amounts of total curcuminoids, total glucuronides, and total phenolic acids, found over a specific time interval in the serum and urine, were calculated by multiplying the correspondent AUC value for 3 L, in the case of serum amounts, and for the volume recorded by the subjects, in the case of urine; the amounts in feces were obtained considering concentration values at specific time points and a mean amount of feces, that is, 150 g.

Statistical analysis was performed using the statistical package SPSS for Windows (version15).

Data were analyzed for differences by one-way analysis of variance (ANOVA) for repeated measures. The concentrations of all monitored compounds were tested for the effect of treatment and of time as factors. When appropriate, the Dunnet t test (2-sided) was used to identify both differences between baseline and results obtained at each time point monitored upon consumption of each type of bread, and the differences between FCB and ECB or ECBB treatment. Statistical significance was set at $p < 0.05$.

RESULTS

Serum. The serum mean concentration−time curves of total curcuminoids, curcuminoid conjugated compounds, and phenolic acids over 24 h following consumption of FCB, ECB, and ECBB are reported in Figure 3.

A different absorption of curcuminoids after consumption of ECB and ECBB compared to FCB was found. Pharmacokinetic data are reported in Table 3. Following FCB, curcuminoids peaked at 30 min with a C_{max} of 1.59 ± 0.28 nmol/L, while following ECB and ECBB t_{max} of 2 and 4 h, with C_{max} of 3.36 ± 0.36 and 2.13 ± 0.39 nmol/L, respectively, were recorded. Serum concentrations of curcuminoids over 6 h after consumption of bread with encapsulated ingredients were always higher than those after consumption of bread with the free ingredient. ECB consumption was determined at 1 and 2 h serum curcuminoid concentrations higher than ECBB ($p < 0.05$ for ECB and ECBB vs FCB and for ECB vs ECBB). The consumption of the second portion of bread at 10 h guaranteed at 24 h (after 14 h) a serum concentration of curcuminoids higher than baseline. Following ECB, that concentration was higher than after FCB (0.93 ± 0.22 nmol/L vs 0.48 ± 0.17 nmol/L, $p < 0.05$).

The curcumin conjugated metabolites found in serum, curcumin glucuronide and hexahydrocurcumin-glucuronide, were at concentrations 10-fold lower than parental curcuminoids. Interestingly, after consumption of encapsulated ingredients, the concentration peaks were anticipated as compared to the free ingredient (30 min and 1 h vs 2 h). In accordance with curcuminoids, also AUC_{0-6} of conjugated compounds were higher after

dx.doi.org/10.1021/jf204517k | J. Agric. Food Chem. 2012, 60, 3357−3366

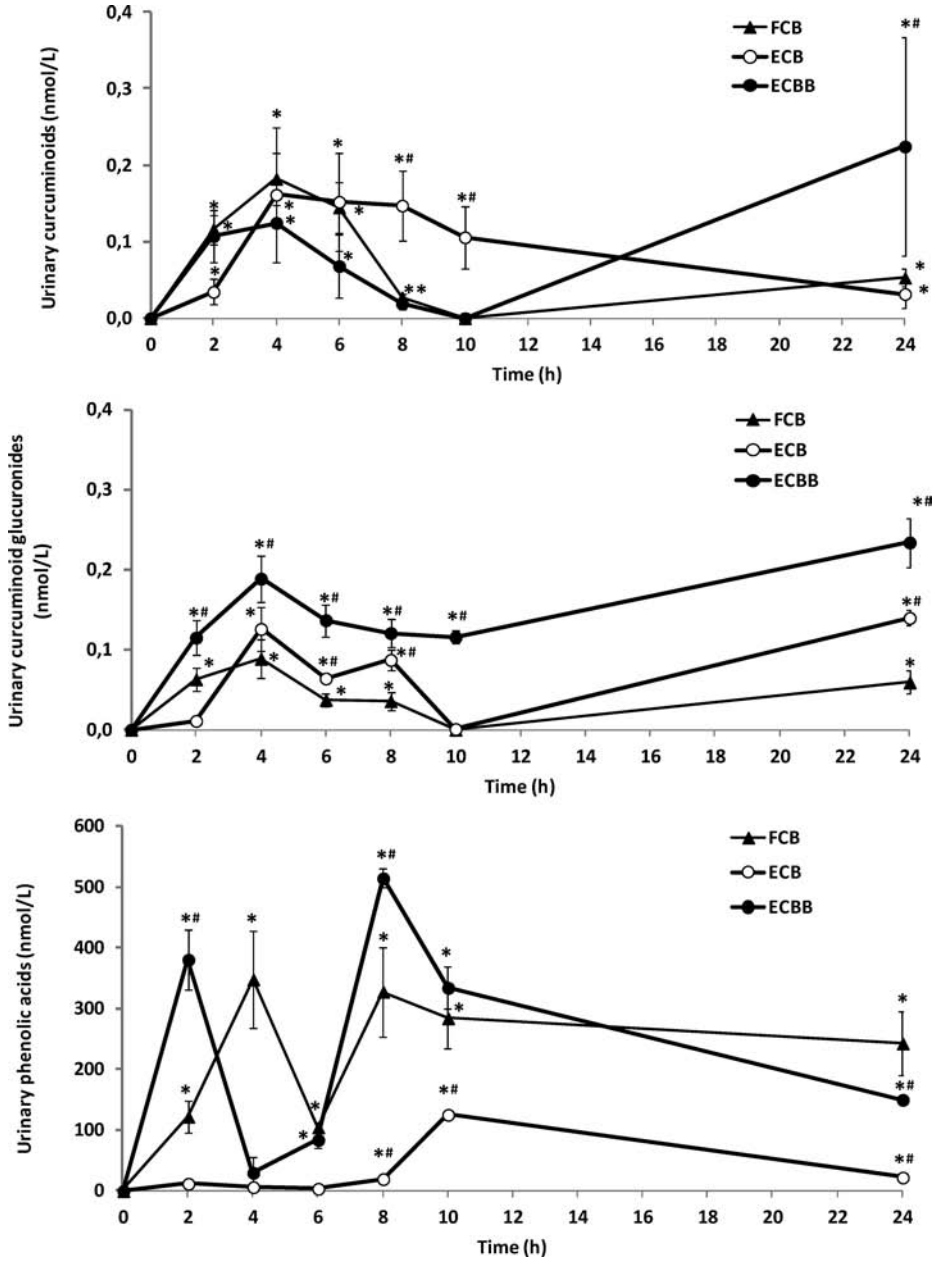

Figure 4. Urine concentration−time curves of total curcuminoids, curcuminoid glucuronides, and phenolic acids following consumption of FCB, ECB, and ECBB. *$p < 0.05$ versus baseline; #$p < 0.05$ versus FCB.

ECBB or ECB than after the FCB (0.32 ± 0.20 nmol·h/L and 0.55 ± 0.39 nmol·h/L vs 0.15 ± 0.06 nmol·h/L, respectively). No glucuronides were found in serum at 24 h.

Following the consumption of all types of bread, the phenolic acids retrieved in serum were ferulic and chlorogenic acid, while vanillic acid was found only after ECBB. Their appearance in serum was already at 30 min and all peaked between 30 min and 2 h; after FCB a double peak at 4 h was recorded. Surprisingly, C_{max} after ECBB was almost 3-fold and 10-fold higher than that after FCB and ECB, respectively (11.43 ± 2.13 nmol/L vs 4.06 ± 1.11 nmol/L and 1.27 ± 0.10 nmol/L, respectively; $p < 0.05$). Measure of AUC_{0-6} of total phenolic compounds demonstrated that the amount of phenolic acids in the bloodstream following ECBB consumption was about 2.6-fold higher than FCB and even 7-fold higher than that following ECB (34.5 ± 19.3 nmol·h/L vs 13.3 ± 6.5 nmol·h/L and 3.3 ± 0.5 nmol·h/L, respectively). In all cases, ferulic acid was the most abundant phenolic acid retrieved in serum, always contributing ∼75% of the total. The repeated consumption of FCB and not that of the two encapsulated bread determined a 24 h serum concentration of phenolic compounds significantly higher than baseline (1.0 ± 0.2 nmol/L).

Urines. Figure 4 reports urinary mean concentration−time curves of total curcuminoids, curcuminoid conjugated compounds, and phenolic acids over 24 h following the consumption of FCB, ECB, and ECBB.

Urinary excretion of curcuminoids began 2 h after consumption of all types of bread. Although AUC_{0-10} of urinary curcuminoids were not significantly different (0.94 ± 0.57 nmol·h/L, 1.10 ± 0.47 nmol·h/L, and 0.64 ± 0.27 nmol·h/L after FCB, ECB, and ECBB, respectively), the excretion kinetics showed a different trend among treatments, as detailed in Table 4.

dx.doi.org/10.1021/jf204517k | J. Agric. Food Chem. 2012, 60, 3357−3366

Table 4. Urinary Pharmacokinetic Data (Mean ± SEM)[a]

	C_{max} (nmol/L)			t_{max} (h)			AUC_{0-10} (nmol·h/L)			AUC_{10-24} (nmol·h/L)		
	FCB	ECB	ECBB	FCB	ECB	ECBB	FCB	ECB	ECBB	FCB	ECB	ECBB
curcuminoids												
curcumin	0.04 ± 0.02	0.16 ± 0.09	0.125 ± 0.05	2–4	2–4	2–4	0.14 ± 0.1	1.1 ± 0.47	0.64 ± 0.27	n.d.	0.96 ± 0.41	1.57 ± 1.00
desmetoxy-curcumin	0.15 ± 0.08	n.d.	n.d.	2–4	n.d.	n.d.	0.78 ± 0.46	n.d.	n.d.	0.37 ± 0.24	n.d.	n.d.
bisdesmethoxy-curcumin	n.q.	n.d.	n.d.	n.q.	n.d.	n.d.	n.q.	n.d.	n.d.	n.q.	n.d.	n.d.
total curcuminoids	0.18 ± 0.10	0.16 ± 0.09	0.125 ± 0.05	2–4	2–8	2–4	0.94 ± 0.57	1. 1 ± 0.47	0.64 ± 0.27	0.37 ± 0.24	0.96 ± 0.41	1.57 ± 1.00
glucuronides												
curcumin-glucuronide	0.04 ± 0.03	n.q.	0.06 ± 0.03	2–4	n.q.	2–4	0.17 ± 0.1	n.q.	0.35 ± 0.17	0.03 ± 0.01	n.q.	0.57 ± 0.22
hexahydrocurcumin-glucuronide	0.05 ± 0.02	0.12 ± 0.05	0.13 ± 0.03	2–4	2–4	2–4	0.28 ± 0.14	0.50 ± 0.14	0.89 ± 0.2	0.38 ± 0.19	0.65 ± 0.00	1.88 ± 0.31
total glucuronides	0.09 ± 0.05	0.13 ± 0.05	0.20 ± 0.06	2–4	2–4	2–4	0.45 ± 0.24	0.58 ± 0.17	1.24 ± 0.37	0.41 ± 0.20	0.99 ± 0.13	2.45 ± 0.53
phenolic acids												
ferulic acid	65.7 ± 37.5	26.10 ± 4.389	67.42 ± 22.34	6–8	8–10	6–8	323.7 ± 190.8	50.59 ± 16.61	217.0 ± 81.60	159.3 ± 53.46	334.4 ± 74.24	521.8 ± 185.8
vanillic acid	281.5 ± 203.5	79.38 ± 18.61	325.9 ± 41.80	2–4	8–10	6–8	1525 ± 1003	79.38 ± 18.61	1320 ± 362.8	1752 ± 1312	558.5 ± 191.9	1340 ± 140.3
diHPA	145.1 ± 81.84	16.44 ± 0.00	4.29 ± 0.00	10–24	8–10	6–8	238.9 ± 79.44	16.44 ± 0.00	14.73 ± 22.02	1776 ± 782.1	115.1 ± 0.00	0.00 ± 0.00
HPP	n.d.	17.83 ± 4.22	n.d.	n.d.	6–8	n.d.	n.d.	61.42 ± 23.64	n.d.	n.d.	23.76 ± 15.46	n.d.
HPA	n.d.	n.d.	218.1 ± 116.4	n.d.	n.d.	8–10	n.d.	n.d.	799.7 ± 520.4	n.d.	n.d.	1527 ± 815.0
total phenolic acids	347.7 ± 238.8	125.3 ± 25.2	514.4 ± 64.1	2–4	8–10	6–8	2088 ± 1274	207.8 ± 58.85	2351 ± 986.9	3688 ± 2142	1031 ± 281.6	3389 ± 1141

[a] n.d., not detected; n.q., not quantifiable.

Curcuminoid concentration peaked within 4 h and was absent 10 h after FCB and ECBB consumption, while it reached a maximum concentration at 4 h and maintained this plateau concentration up to 10 h after ECB. Concentrations higher than baseline were still found 14 h after consumption of the second portion of each type of bread.

Curcumin-glucuronides and hexahydrocurcumin-glucuronides were the conjugated compounds retrieved in urine (the same compounds found in serum). In the urine, conjugated compounds and parental curcuminoids were present at the same concentration range. Similarly to parent compounds, most of glucuronides peaked between 2 and 4 h after all types of bread, but they were excreted within 10 h only after FCB and ECB. Over 10 h from consumption of ECBB, glucuronides concentration was always significantly higher than those found after FCB and ECB (AUC_{0-10} being 1.24 ± 0.37 nmol·h/L vs 0.45 ± 0.24 nmol·h/L and 0.58 ± 0.17 nmol·h/L, respectively, $p < 0.05$). The consumption of the second portions of breads enriched with encapsulated ingredients determined an overnight excretion significantly higher than that found with FCB (AUC_{10-24} being 0.99 ± 0.13 nmol·h/L after ECB and 2.45 ± 0.53 nmol·h/L after ECBB vs 0.41 ± 0.20 nmol·h/L for FCB).

Phenolic acids found in urine after consumption of all types of bread were ferulic, vanillic acid, and diHPA, while HPP and HPA were also found after ECB and ECBB, respectively. The concentrations of phenolic acids were 1000-fold higher than the other urinary metabolites and 50−100-fold higher than serum phenolic acids. In accordance with serum data, ECB determined the lowest urinary excretions of phenolic acids: they appeared in urine 6 h after consumption peaking at 10 h with a C_{max} of 125.6 ± 22.9 nmol/L. On the contrary, phenolic acid excretion after ECBB paralleled that after FCB: a first excretion peak within 4 h after consumption of bread and another peak over the next 4 h, with C_{max} ranging between 347.7 ± 79.6 and 514.4 ± 16.0 nmol/L. AUC_{0-10} of phenolic acids following ECB (0.21 ± 0.06 μmol·h/L) was 10-fold lower than those measured upon consumption of FCB (2.1 ± 1.3 μmol·h/L) and ECBB (2.4 ± 1.0 μmol·h/L). Interestingly, individual phenolic acids differently contributed to total amount excreted upon each experimental condition. After FCB, vanillic acid was the most abundant phenolic acid being 73% of total phenolic acids, while ferulic acid contributed by 16% and diHPA by 11%. Encapsulation deeply modified the pattern of phenolic acids retrieved eliciting a significant amount of HPP: after ECB consumption, the percentages of excretion of ferulic acid (24%) and diHPA (8%) were, respectively, slightly higher and lower than those found after FCB, while the remaining part was almost equally represented by vanillic acid (38%) and HPP (30%). The presence of piperine, quercetin, and genistein in the encapsulated material further modified the pattern: in fact, after ECBB, as compared to ECB, an increase of vanillic acid (56%) versus a reduction of ferulic acid (9.2%) and diHPA (0.6%) as well as the excretion of HPA (34%) instead of HPP were found.

Feces. Concentrations of monitored compounds in fecal samples collected the day after consumption of each type of bread are reported in Table 5. Data showed that ECB consumption increased about 6-fold the amount of curcuminoids in the feces with respect to FCB. On the contrary, following ECBB consumption, trace amounts of curcuminoids and the highest amount of phenolic acids were found, the latter being even 4-fold higher than after ECB.

dx.doi.org/10.1021/jf204517k | J. Agric. Food Chem. 2012, 60, 3357−3366

DISCUSSION

Previous bioavailability studies reported that following consumption of 2−12 g of curcumin administered in form of capsules as pharmaceutical preparations, serum C_{max} was always around 130 nmol/L in healthy subjects,[4,15,16] while concentrations around 10 nmol/L were found in patients ingesting 3.6 g/die of curcumin.[17] Our data, showing curcuminoid serum concentrations always lower than 4 nmol/L, suggested that curcumin is less bioaccessible when included in bread probably due to a strong interaction of the compound with the processed food matrix. Encapsulation increased the bioavailability of curcuminoids from bread both upon consumption of a single and a double portion, while coencapsulation of curcuminoids with piperine, quercetin and genistein, slightly reduced this effect. In fact, over 10 h from the first consumption of ECB and ECBB, total curcuminoids were, respectively, 7.3- and 4.6-fold higher than after FCB, with ECB leading to a 63% higher serum amount of curcuminoids than ECBB (ECB > ECBB ≫ FCB) (Table 6).

The in vivo formation of several phenolic acids following consumption of curcuminoids was demonstrated for the first time in this study. In fact, the amount of phenolic acids in bread was very negligible as compared to the added curcuminoids (less than 4 mg/100 g as compared to 1 g/100 g, with the ratio phenolic acids:curcuminoids in bread being 1:270 w:w), and it was in equal proportion in all types of bread (without explaining the differences we found among treatments). Thus, we assumed that phenolic acids in biological samples might mainly derive from curcumin, due to its known chemical instability and potential in vivo metabolism. Curcumin degradation is pH-dependent (faster at neutral-basic conditions), due to oxidative mechanisms and leading to the formation of *trans*-6-(4′-hydroxy-3′-methoxy-phenyl)-2,4-dioxo-5-hexenal, vanillin, ferulic acid, and feruloyl methane.[18−21] In particular, it has been shown that when curcumin was incubated in 0.1 M phosphate buffer (pH 7.2, 37 °C), 90% of it decomposed within 30 min and 20% decomposed within 1 h by incubation in cell culture or in human blood leading to increase of vanillin.[19] Thus, it can be hypothesized that in the intestine, most of the curcumin may decompose at neutral pH before absorption, and a minor part may be even degraded in intestinal mucosa and in the bloodstream. When curcuminoids are microencapsulated, they are protected from intestinal degradation, thus increasing their amount in blood in the original chemical form. On the other hand, the concomitant presence in the intestine of curcuminoids and the three bioactive compounds as in ECBB did not influence intestinal and hepatic glucuronidation of curcumin, but it promoted phenolic acid formation. This might be caused by the competitive absorption between curcumin and the other compounds at the intestinal mucosa level leading to a delayed curcumin absorption and a consequent increased degradation rate in the intestinal lumen. This hypothesis is consistent with a recent study showing a faster and a more efficient absorption of piperine than curcumin in rats.[22] Moreover, recent studies highlighted the influence of dietary piperine, quercetin, and genistein on drug absorption by

Table 5. Concentrations of Curcuminoids, Curcuminoid Glucuronides, and Phenolic Acids Found in Fecal Samples Collected the Day after Consumption of Each Type of Bread[a]

	FCB	ECB	ECBB
total curcuminoids	0.59 ± 0.38	3.49 ± 2.13	0.01 ± 0.01
curcumin	0.29 ± 0.18	0.83 ± 0.46	
desmetoxycurcumin	0.30 ± 0.19	2.65 ± 1.67	0.01 ± 0.01
bisdesmetoxycurcumin		0.01 ± 0.01	
total glucuronides			
curcumin-glucuronide			
hexahydrocurcumin-glucuronide			
total phenolic acids	0.73 ± 0.47	0.49 ± 0.32	2.04 ± 1.27
chlorogenic acid			
ferulic acid		0.01 ± 0.00	0.01 ± 0.01
vanillic acid	0.69 ± 0.45		2.03 ± 1.26
di-HPA		0.02 ± 0.01	
HPA		0.46 ± 0.30	
HPP	0.03 ± 0.02		

[a]Data are mean ± S.E. pmol/g.

Table 6. Mean Amount (nmol) of Total Curcuminoids, Curcuminoid Glucuronides, and Phenolic Acids Found in Serum, Urine, and Feces Following Consumption of One Portion of Each Type of Bread or over 24 (Following Consumption of Two Portions of Each Type of Bread)

	bioavailability upon single portion					bioavailability upon double portion				
	total curcuminoids (nmol)	total glucuronides (nmol)	phenolic acids (nmol)	total (nmol)	% dose ingested	total curcuminoids (nmol)	total glucuronides (nmol)	phenolic acids (nmol)	total (nmol)	% dose ingested
FCB										
serum	5.00	0.45	39.85	2513	0.09				5418	0.10
urine	1.15	0.33	2466			1.45	0.64	5416		
feces						0.59		0.73		
sum	6.15	0.78	2506			1.45	0.64	5416		
ECB										
serum	36.34	0.97	9.95	957.5	0.04				1170	0.02
urine	1.65	0.75	907.8			2.45	1.28	1166		
feces						3.49		0.01		
sum	37.99	1.72	917.8			2.45	1.28	1166		
ECBB										
serum	23.00	1.65	103.4	4648					6209	0.11
urine	0.73	1.31	4518		0.17	2.18	2.83	6204		
feces						0.49		2.04		
sum	23.74	2.96	4622			2.18	2.83	6204		

dx.doi.org/10.1021/jf204517k | J. Agric. Food Chem. 2012, 60, 3357−3366

several mechanisms (i.e., modification of absorptive sites on mucosa or interaction with P-glycoproteins),[23−26] other than modification of first pass metabolism.[6,10] Thus, in the case of ECBB, we hypothesize that phenolic acids deriving from curcumin and from coingested piperine, quercetin, and genistein biotransformation[27] might be also absorbed more efficiently.

On the other hand, the prevalence of ferulic acid in serum was clearly explainable from chemical instability of curcumin, while vanillic acid might be formed in liver by enzymatic oxidation of vanillin.[28] The metabolism by gut microflora of not absorbed curcuminoids[20] and of compounds formed in the upper intestine and successive absorption of metabolites through the colon could explain the presence of phenolic acids in biological samples collected at 24 h. The active role of intestinal microbiota on metabolism of curcuminoids was consistent with a recent study where a microbial enzyme isolated from human feces able to convert curcumin in dihydrocurcumin and tetrahydrocurcumin was isolated.[29]

In conclusion, in this study, the bioavailability and biotransformation of curcuminoids present in free and microencapsulated form in a processed food such as bread was elucidated. The concentration of curcuminoids in serum following consumption of the new types of bread was much lower than that reported from curcuminoid supplements. The use of encapsulated ingredients can protect curcumin by in vivo biotransformation, thus increasing its circulating concentration as compared to the free ingredient. On the other hand, coingestion of piperine, quercetin, and genistein did not increase curcuminoid bioavailability in their original form but increased their biotransformation in phenolic acids. It was demonstrated for the first time that phenolic acids (mainly ferulic acid and vanillic acid) are the major metabolites following consumption of curcuminoids, thus envisaging their potential contribution to the biological properties recognized to curcuminoids despite their generally low bioavailability.

AUTHOR INFORMATION

Corresponding Author

*Tel.: +39 0812539356. Fax: +39 0817762580. E-mail: paola.vitaglione@unina.it.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This study was conducted in the frame of the BIOCURCUMIN project funded by the Italian Ministry of Foreign affairs and by Israeli MATIMOP by the NANOFOODS project funded by the European Commission (Project no: 222006) and by the PON project ENCAPSULATION funded by the Italian Ministry of Research (MIUR).

ABBREVIATIONS USED

FCB, free curcumin bread; ECB, encapsulated curcumin bread; ECBB, encapsulated curcumin plus other polyphenols bread; HPA, 4-hydroxyphenylacetic acid; diHPA, 3,4-dihydroxyphenylacetic acid; HPP, 3-(4-hydroxyphenyl)propionic acid; NSAID, non-steroidal anti-inflammatory drugs

REFERENCES

(1) Singh, S.; Aggarwal, B. B. Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane). *J. Biol. Chem.* **1995**, *270*, 4995−5000.

(2) Pari, L.; Tewas, D.; Eckel, J. Role of curcumin in health and disease. *Arch. Physiol. Biochem.* **2008**, *114*, 127−149.

(3) Lao, C.; Ruffin, M.; Normolle, D.; Heath, D.; Murray, S.; Bailey, J. Dose escalation of a curcuminoid formulation. *BMC Complementary Altern. Med.* **2006**, *6*, 10−15.

(4) Cheng, A. L.; Hsu, C. H.; Lin, J. K.; Hsu, M. M.; Ho, Y. F.; Shen, T. S.; Ko, J. Y.; Lin, J. T.; Lin, B. R.; Ming-Shiang, W.; Yu, H. S.; Jee, S. H.; Chen, G. S.; Chen, T. M.; Chen, C. A.; Lai, M. K.; Pu, Y. S.; Pan, M. H.; Wang, Y. J.; Tsai, C. C.; Hsieh, C. Y. Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. *Anticancer Res.* **2001**, *21*, 2895−2900.

(5) Bansal, S. S.; Goel, M.; Aqil, F.; Vadhanam, M. V.; Gupta, R. C. Advanced drug-delivery systems of curcumin for cancer chemoprevention. *Cancer Prev. Res.* **2011**, *4*, 1158−1171.

(6) Shoba, G.; Joy, D.; Joseph, T.; Majeed, M.; Rajendran, R.; Srinivas, P. S. Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. *Planta Med.* **1998**, *64*, 353−356.

(7) Shaikh, J.; Ankola, D. D.; Beniwal, V.; Singh, D.; Kumar, M. N. Nanoparticle encapsulation improves oral bioavailability of curcumin by at least 9-fold when compared to curcumin administered with piperine as absorption enhancer. *Eur. J. Pharm. Sci.* **2009**, *37*, 223−230.

(8) Lambert, J. D.; Kwon, S. J.; Ju, J.; Bose, M.; Lee, M. J.; Hong, J.; Hao, H.; Yang, C. S. Effect of genistein on the bioavailability and intestinal cancer chemopreventive activity of (-)-epigallocatechin-3-gallate. *Carcinogenesis* **2008**, *29*, 2019−2024.

(9) Bansal, T.; Awasthi, A.; Jaggi, M.; Khar, R. K.; Talegaonkar, S. Pre-clinical evidence for altered absorption and biliary excretion of irinotecan (CPT-11) in combination with quercetin: possible contribution of P-glycoprotein. *Life Sci.* **2008**, *83*, 250−259.

(10) Choi, J. S.; Piao, Y. J.; Kang, K. W. Effects of quercetin on the bioavailability of doxorubicin in rats: role of CYP3A4 and P-gp inhibition by quercetin. *Arch. Pharm. Res.* **2011**, *34*, 607−613.

(11) Fogliano, V.; Vitaglione, P. Functional foods: planning and development. *Mol. Nutr. Food Res.* **2005**, *49*, 256−262.

(12) Marczylo, T. H.; Verschoyle, R. D.; Cooke, D. C.; Morazzoni, P.; Steward, W. P.; Gescher, A. J. Comparison of systemic availability of curcumin with that of curcumin formulated with phosphatidylcholine. *Cancer Chemother Pharmacol.* **2007**, *60*, 171−177.

(13) Urpi-Sarda, M.; Monaga, M.; Khan, N.; Llorach, R.; Lamuela-Raventòs, R. M.; Jàuregui, O.; Estruch, R.; Izquierdo-Pulido, M.; Andrés-Lacueva, C. Targeted-metabolic profiling of phenolics in urine and plasma after regular consumption of cocoa by liquid chromatography-tandem mass spectrometry. *J. Chromatogr., A* **2009**, *1216*, 7258−7267.

(14) Vitaglione, P.; Donnarumma, G.; Napolitano, A.; Galvano, F.; Gallo, A.; Scalfi, L.; Fogliano, V. Protocatechuic acid is the major human metabolite of cyanidin-glucosides. *J. Nutr.* **2007**, *137*, 2043−2048.

(15) Antony, B.; Merina, B.; Iyer, V. S.; Judy, N.; Lennertz, K.; Joyal, S. A pilot cross-over study to evaluate human oral bioavailability of BCM-95CG (Biocurcumax), a novel bioenhanced preparation of curcumin. *Indian J. Pharm. Sci.* **2008**, *70*, 445−449.

(16) Vareed, S. K.; Kakarala, M.; Ruffin, M. T.; Crowell, J. A.; Normolle, D. P.; Djuric, Z.; Brenner, D. E. Pharmacokinetics of curcumin conjugate metabolites in healthy human subjects. *Cancer Epidemiol., Biomarkers Prev.* **2008**, *17*, 1411−1417.

(17) Sharma, R. A.; Euden, S. A.; Platton, S. L.; Cooke, D. N.; Shafayat, A.; Hewitt, H. R.; Marczylo, T. H.; Morgan, B.; Hemingway, D.; Plummer, S. M.; Pirmohamed, M.; Gescher, A. J.; Steward, W. P. Phase I clinical trial of oral curcumin: biomarkers of systemic activity and compliance. *Clin. Cancer Res.* **2004**, *10*, 6847−6854.

(18) Oetari, S.; Sudibyo, M.; Commandeur, J. N.; Samhoedi, R.; Vermeulen, N. P. Effects of curcumin on cytochrome P450 and glutathione S-transferase activities in rat liver. *Biochem. Pharmacol.* **1996**, *51*, 39−45.

(19) Wang, Y. J.; Pan, M. H.; Cheng, A. L.; Lin, L. I.; Ho, Y. S.; Hsieh, C. Y.; Lin, J. K. Stability of curcumin in buffer solutions and

characterization of its degradation products. *J. Pharm. Biomed. Anal.* **1997**, *15*, 1867−1876.
(20) Pan, M. H.; Huang, T. M.; Lin, J. K. Biotransformation of curcumin through reduction and glucuronidation in mice. *Drug Metab. Dispos.* **1999**, *27*, 486−494.
(21) Griesser, M.; Pistis, V.; Suzuki, T.; Tejera, N.; Pratt, D. A.; Schneider, C. Autoxidative and cyclooxygenase-2 catalyzed transformation of the dietary chemopreventive agent curcumin. *J. Biol. Chem.* **2011**, *286*, 1114−1124.
(22) Suresh, D.; Srinivasan, K. Tissue distribution & elimination of capsaicin, piperine & curcumin following oral intake in rats. *Indian J. Med. Res.* **2010**, *13*, 682−691.
(23) Prakash, U. N.; Srinivasan, K. Beneficial influence of dietary spices on the ultrastructure and fluidity of the intestinal brush border in rats. *Br. J. Nutr.* **2010**, *104*, 31−39.
(24) Jin, M. J.; Han, H. K. Effect of piperine, a major component of black pepper, on the intestinal absorption of fexofenadine and its implication on food-drug interaction. *J. Food Sci.* **2010**, *75*, 93−96.
(25) Han, H. K. The effects of black pepper on the intestinal absorption and hepatic metabolism of drugs. *Expert Opin. Drug Metab. Toxicol.* **2011**, *7*, 721−729.
(26) Choi, J. S.; Piao, Y. J.; Kang, K. W. Effects of quercetin on the bioavailability of doxorubicin in rats: role of CYP3A4 and P-gp inhibition by quercetin. *Arch. Pharm. Res.* **2011**, *34*, 607−613.
(27) Visioli, F.; Alarcon De La Lastra, C.; Andres-Lacueva, C.; Aviram, M.; Calhau, C.; Cassano, A.; D'archivio, M.; Faria, A.; Favé, G.; Fogliano, V.; Lorach, R.; Vitaglione, P.; Zoratti, M.; Edeas, M. Polyphenols and human health: a prospectus. *Crit. Rev. Food Sci.* **2011**, *51*, 524−546.
(28) Panoutsopoulos, G. I.; Kouretas, D.; Beedham, C. Contribution of aldehyde oxidase, xanthine oxidase, and aldehyde dehydrogenase on the oxidation of aromatic aldehydes. *Chem. Res. Toxicol.* **2004**, *17*, 1368−1376.
(29) Hassaninasab, A.; Hashimoto, Y.; Tomita-Yokotani, K.; Kobayashi, M. Discovery of the curcumin metabolic pathway involving a unique enzyme in an intestinal microorganism. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 6615−6620.