Fluorometric Determination of Curcumin in Yogurt and Mustard

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A method for the fluorometric determination of curcumin in acetonitrile is described. By the normal, synchronous, and synchronous first and second derivative techniques determinations can be made between 0.27 and 1500 ng/mL. A detection limit of 0.08 ng/mL was achieved with the direct and synchronous approach. The method is applied to the determination of curcumin in several yogurt and mustard samples.

The rhizomes of *Curcuma longa* Linn (*Turmeric*) are used as a natural coloring matter in food processing. The rhizomes contain 1–5% curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] and two related demethoxy compounds present in small amounts.

So far, there has been no reason to believe that the consumption of curcumin by man as a part of the diet is associated with any deleterious effects. WHO/FAO has approved specification for curcumin and a temporary acceptable daily intake of 0–0.1 mg/kg body weight (FAO, 1974). However, because of the emphasis on the use of curcumin as a natural food colorant, a number of methods have been developed to measure the total color. The method most widely used for quantification of curcumin is a direct spectrophotometric method (ASTA, 1958), but a lack of reproducibility is observed (Govindarajan, 1980). Also, an intensely colored complex can be developed by reaction with boric acid (Satyanarayana et al., 1969; AOAC, 1970); this test is reported to give inconclusive results because of interference from coextractives (Karasz et al., 1973).

Curcumin exhibits strong fluorescence in organic solvents. A direct method has been developed for detection of curcumin (Karasz et al., 1973). A fluorometric method for quantitative total curcuminoids determination has been proposed with a minimum concentration that could be determined in the order of 5 ng/mL (Wahlstrom and Blennow, 1978); however, no optimization parameters have been realized, and no better analytical conditions are established.

Separation of the curcuminoids is achieved by thin-layer or paper chromatography (Govindarajan, 1980; Tonnesen, 1986; Janssen and Gole, 1984). Quantification was very time-consuming. It was accomplished by scraping off the respective spots, redissolving, centrifuging, and finally reading the absorbance. A more rapid separation for the pigments in turmeric using HPLC with fluorescence detection has been recently reported (Tonnesen and Karlsen, 1983; Rouseff, 1988).

The purpose of this work is to determine the colorant curcumin in food samples by a simple spectrofluorometric technique.

For direct fluorometric determination, the reported advantages (Lloyd and Evett, 1977) of the band-narrowing effect of the synchronous scanning technique can be very valuable (Vo-Dinh, 1978; García Sánchez et al., 1985). On the other hand, a key feature of the fluorescence derivative technique is that broad bands are minimized relative to sharper bands, to an extent which increases with the derivative order. The combination of these approaches is valuable in increasing the sensitivity of the determination of compounds characterized by broad and poorly structured excitation or emission spectra or both (García Sánchez and Cruces, 1984, 1989a–c; Rubio et al., 1985).

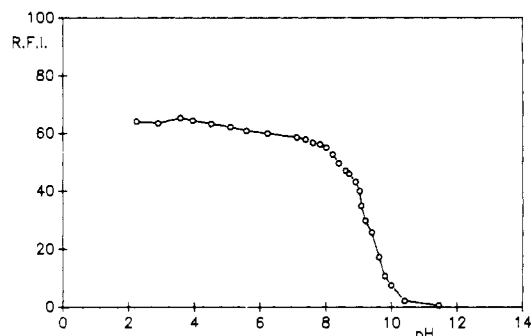

Figure 1. Fluorescence–pH dependence of curcumin in 50% acetonitrile. λ_{ex} = 397 nm; λ_{em} = 508 nm.

Table I. Fluorescent Characteristics of the Curcumin in Different Solvents

solvent	dielectric constant	λ_{ex}	λ_{em}	RFI
1,4-dioxane	2.21 (25 °C)	373–438	492	146
ethyl acetate	6.02 (25 °C)	374–437	492	149
acetic acid	6.15 (20 °C)	396–447	516	45
1-butanol	17.1 (25 °C)	400–455	521	120
acetone	20.7 (25 °C)	370–441	504	147
ethanol	24.3 (25 °C)	392–450	522	76
acetonitrile	37.5 (20 °C)	397–438	508	151
N,N-dimethylformamide	37.6 (25 °C)	390–457	513	42

Table II. Influence of Water Percentage

% water	RFI	% water	RFI
90	2	40	8
80	3	30	10
70	4	20	14
60	5	10	22
50	6	0	98

EXPERIMENTAL METHODS

Apparatus. Fluorescence measurements were obtained with a Perkin-Elmer LS-5 luminescence spectrometer equipped with a 9.9-W xenon discharge lamp pulsed at line frequency and F/3 Monk-Gilleon type monochromators. Standard 1-cm quartz cells were used. The spectrometer was operated in the computer-controlled mode with an RS232C serial interface to a Perkin-Elmer Model 3600 data station. Instrumental control and data collection were achieved with the Perkin-Elmer luminescence software (PECLS II) modified as described in García Sánchez et al. (1988). An Epson FX-800 printer was used to record spectra.

Reagents and Solvents. Curcumin was obtained from Sigma Chemical Co. (Ref 1386). No percentage of purity is labeled in the commercial product, specified as crystalline. Stock solutions (2.175×10^{-3} M) were prepared in acetonitrile and stored in the

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Table III. Characteristics of the Analytical Methods

method	s_B RFI	C_L, ng/mL	dynamic range, ng/mL	s_S RFI	S_A, ng/mL	amt taken, ng/mL	amt found, ng/mL	error,[a] %	RSD,[b] %
direct	0.29	0.08	0.27–1500	3.60	0.34	5.00	4.86	5.27	7.00
				1.70	1.35	50.00	50.41	2.02	2.68
				1.43	0.03	500.00	508.00	4.43	5.88
synchronous	0.34	0.08	0.26–1500	5.79	0.44	5.00	4.89	6.78	9.00
				2.18	1.41	50.00	51.58	2.06	2.71
				1.75	0.03	500.00	516.00	4.35	5.77
synchronous first derivative	0.62	0.16	0.53–1500	5.55	0.47	5.00	5.48	6.46	8.58
				2.44	1.78	50.00	52.52	2.52	3.41
				1.53	0.03	500.00	523.00	4.35	5.77
synchronous second derivative	0.91	0.11	0.37–1500	11.59	0.47	5.00	4.83	7.33	9.73
				4.43	1.54	50.00	52.71	2.20	2.92
				3.26	0.03	500.00	523.00	4.35	5.77

[a] Relative error = $100ts/\bar{x}n^{1/2}$. [b] RSD = $100s/\bar{x}$.

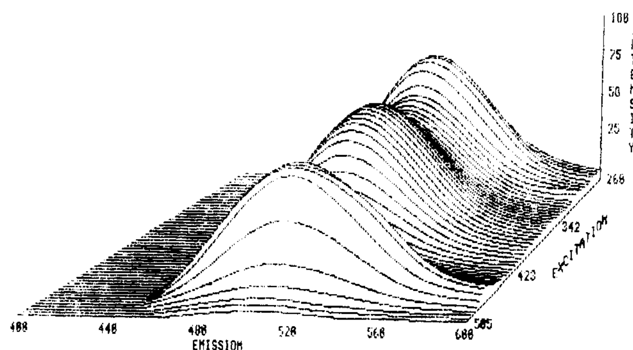

Figure 2. Three-dimensional emission spectrum of curcumin 2.44×10^{-5} M.

dark in amber bottles at 4 °C. Dilute solutions were prepared daily from these solutions. The water used was both distilled and demineralized. All solvents used were of analytical reagent grade.

Analytical Procedure. An aliquot of curcumin solution is placed in a 10-mL standard flask to give a final concentration between 0.27 and 1500 ng/mL and diluted to volume with acetonitrile. The relative fluorescence intensity is measured at λ_{ex} = 397 nm and λ_{em} = 508 nm using slit widths of 5 nm, against a reagent blank. First and second derivatives (wavelength increment of 10 nm) of the synchronous spectrum ($D\lambda$ = 80 nm) are recorded with a response time of 4 s and a scan speed of 480 nm/min. The derivative values are measured as the vertical distance from the peak to the trough and from the right-hand peak to the trough, for the first and second derivatives, respectively.

Extraction Procedure. Curcumin is extracted from the sample, 2.0 g of yogurt or 0.3 g of mustard, by adding 30 mL of acetonitrile and shaking for 5 min. The mixture is filtered through Whatman paper, and the filtrate is transferred to a 50-mL standard flask and diluted to volume with acetonitrile. This solution is analyzed according to the proposed analytical procedure.

Recoveries. To determine recoveries, yogurt and mustard samples were prepared and spiked with curcumin to obtain in the extract a total sample value in the range of the standard curves for curcumin.

RESULTS AND DISCUSSION

To determine the effect of pH on the fluorescence of curcumin, a fluorometric titration of curcumin (2.44×10^{-5} M in 50% v/v acetonitrile) is carried out. The results are shown in Figure 1. In acidic media, below pH 8, a yellow fluorescence appears. In basic media, a red nonfluorescent form is shown. The pK_a value for curcumin (pK_a = 9.12 ± 0.34) was calculated from the fluorometric titration by application of the equation recommended by Rosenberg et al. (1978).

To evaluate solute–solvent interaction and select an adequate solvent for fluorometric determination of curcumin, the fluorescence spectra of curcumin solutions (2.44×10^{-5} M) in solvents of different polarities and hydrogen-bonding capacities were recorded. The most relevant characteristics found are summarized in Table I. The excitation spectrum in each solvent is characterized by a wide and poorly structured band. A blue shift is observed in λ_{ex} and λ_{em} with increasing solvent dielectric constant. The largest values of the relative fluorescence intensity (RFI) are obtained in dioxane, ethyl acetate, acetone, and acetonitrile; the minimum value is obtained in *N,N*-dimethylformamide. So with the use of acetonitrile as solvent medium sensitivity will increase.

Curcumin in aqueous solutions is exposed to degradative reactions (Tonnesen and Karlsen, 1985). To obtain optimum stability of the preparations, the pH should be maintained below 7. However, in this pH region the solubility of curcumin in aqueous media is low. The effect of water percentage on fluorescence readings of curcumin solutions (1.22×10^{-5} M) in acetonitrile is reported in Table II. The fluorescence intensity increases exponentially when the acetonitrile percentage is increased to 100%. The fluorescence intensity of curcumin solutions in 50% acetonitrile–water at acidic pH (pH 2.35) is lower that the one obtained with a 100% acetonitrile percentage. Hence, the method must be applied in anhydrous acetonitrile medium. Tonnesen (1986) has reported half-lives for the overall degradation of curcumin in acetonitrile after irradiation with light (400–750 nm) between 6.3 h for 2×10^{-4} M solutions and 1.6 h for 8×10^{-6} M solutions. These are times large enough to realize fluorometric analysis of curcumin. It has been confirmed that acetonitrile solutions of curcumin (2.44×10^{-5} M) gave constant fluorescence readings for at least 1 h.

Although through the monochromators used in the spectrofluorometer very low intensities are transmitted and photochemical degradation is not probably, the influence of slit widths on the emission spectrum of 4.10×10^{-5} M curcumin has been studied, because of the photochemical instability reported. Excitation and emission slit widths between 2.5 and 20 nm have been changed and the RFI measured. We have selected slit widths of 5 nm; in these conditions curcumin solutions are not affected by photodecomposition, and maximum fluorescence is achieved.

The effect of reagent concentration was tested and showed a linear increase in signal up to 3×10^{-4} M curcumin concentration and further decrease at higher concentrations.

The total luminescence emission spectra of 2.44×10^{-5} M curcumin under the optimal experimental conditions is represented as a three-dimensional plot in Figure 2. Each horizontal slice represents a separate emission scan with a fixed excitation wavelength. A slice parallel to the excitation axis represents an excitation spectrum. It can

Table IV. Determination of Curcumin in Yogurt and Mustard

sample	curcumin found in the extract,[a] ng/mL			
		synchronous		
	direct		first derivative	second derivative
lemon yogurt, Yoplait	88.69 ± 1.12	90.83 ± 2.00	85.59 ± 1.76	86.21 ± 1.69
lemon yogurt, Dannon	86.04 ± 0.55	85.15 ± 0.69	81.39 ± 0.88	82.42 ± 0.95
pineapple yogurt, Dannon	85.70 ± 0.34	86.23 ± 1.26	84.90 ± 2.52	84.54 ± 2.42
mustard in bulk	0.29 ± 0.020	0.29 ± 0.018	0.28 ± 0.021	0.28 ± 0.020
mustard, McCormick	0.89 ± 0.025	0.89 ± 0.030	0.87 ± 0.023	0.90 ± 0.026

[a] Mean ± standard deviation (three measurements).

be observed that there is one emission maximum at 508 nm and a wide excitation band with maximum at 390–438 nm.

For direct determination of curcumin, the advantages of the synchronous and synchronous derivative scanning were exploited. For selection of the appropriate wavelength scanning interval ($D\lambda$), various synchronous spectra from $D\lambda$ = 40 nm to $D\lambda$ = 130 nm (Stokes shift) were recorded. The better signal-to-noise ratio and the narrow peak half-width can be obtained by using $D\lambda$ = 80 nm.

The variables to be optimized when the derivative technique is applied to synchronous spectra are (García Sánchez et al., 1985) the wavelength increment, response time, t_r, and scan speed. The highest intensity and signal-to-noise ratio were obtained with a wavelength increment of 10 nm, t_r = 4 s, v = 480 nm/min.

Calibration Graphs, Sensitivity, and Precision. The calibration graphs were prepared by plotting the normal, synchronous, and synchronous first and second derivative values against standard curcumin between 1 and 1500 ng/mL. The detection limit and determination limit defined by IUPAC (Long and Winefordner, 1983), are $C_L = 3s_B/m$ and $C_Q = 10s_B/m$ (s_B is the standard deviation of the blank signals and m is the slope of the calibration curve). C_Q is employed to establish the lower limit of the dynamic range. The sensitivities of the methods are reported as the analytical sensitivity $S_A = s_S/m$, where s_S is the standard deviation of analytical signal (Navas Díaz and Sánchez, 1984). Table III presents the results obtained, together with other details about the precision and accuracy of the methods.

Analysis of Yogurt and Mustard. Samples of mustard and yogurt acquired in a commercial food establishment were separately submitted to the extraction procedure and further analyzed in triplicate according to the proposed method. The results are indicated in Table IV.

To evaluate the interference of other colorants, we have compared the results obtained for yogurt of the same commercial brand but different flavor (lemon and pineapple); in the latter carotene is also present. Yogurt samples of the same flavor (lemon) but different commercial brands have also been analyzed. In all yogurt samples the curcumin found was similar.

Recovery Assay. To evaluate the extraction procedure and the accuracy of the method, a recovery assay was realized. To a pineapple yogurt and a mustard sample was added curcumin in such amount that in the extract were found 10 ng/mL and 0.5 μg/mL, respectively, additional to that initially present in both samples. The results showed that 99.59 ± 1.75 ng/mL curcumin was found in the extract from yogurt, which gave a recovery of 105.19 ± 2.09%. For mustard 1.31 ± 0.02 μg/mL was found in the extract, which gave a recovery of 94.24 ± 1.24%.

CONCLUSION

A fluorometric method has been developed for determination of curcumin colorant by using its characteristic intrinsic fluorescence in acetonitrile. The method is sensible, simple, rapid, and reproducible.

Precise determination of concentrations in yogurt and mustard at the nanogram/milliliter level were made. Recovery values were measured to evaluate the extraction procedure and accuracy of the method and gave satisfactory results.

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