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Interaction of Curcumin and Bixin with β-Cyclodextrin: Complexation Methods, Stability, and Applications in Food

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ABSTRACT: This work aimed to compare methods for the formation of complexes of bixin and curcumin with β-cyclodextrin (β-CD) and to evaluate the stability of the complexes formed by these methods and their food applications. The stoichiometric relationship between curcumin and β-CD was 1:2 and that between bixin and β-CD was 1:1. Curcumin-β-CD and bixin-β-CD complexes formed by kneading, coprecipitation, and simple mixing were evaluated by differential scanning calorimetry (DSC), thermogravimetry analysis (TGA), or nuclear magnetic resonance (NMR-H). For both curcumin and bixin, the best method of complexation was coprecipitation. Complexation of colorants with β-CD promoted an intensification of color and increased water solubility; however, stabilization in the presence of light occurred only for bixin. Application of curcumin-β-CD in cheese and yogurt and bixin-β-CD in the curd did not alter the initial characteristics of the products, which were sensorialy well accepted. Therefore, the complexation of these natural colorants with β-CD favors their use in low-fat foods, broadening the field of industrial application.

KEYWORDS: β-Cyclodextrin, curcumin, bixin, complexation methods, stability

INTRODUCTION

Annatto and curcumin extracts, as well as blends of these two dyes, are among the most widely used natural colorants in various branches of industry. *Annatto* is a carotenoid with shades of yellow and red and is used in much of the canned food industry as well as in dairy products, beverages, sausages, meats, and candies.[1] It is estimated that in 2008 the world production of *annatto* was 17,500 tons; of this amount, Brazil is responsible for approximately 12,000 tons; however, only 25% of total industrial *annatto* seeds are used for the preparation of extracts.[2,3] India is the world's largest producer of *Curcuma longa* tubercles; estimates of its production range from 250 to 300 tons/year. Most of this tubercle is consumed in the form of seasoning; only a small fraction (1−1.5 tons) is converted to extract.[1]

Urucum (bixin) (*annatto* or *Bixa orellana*), a small tree of the family *Bixaceae*, is native to South and Central America and is widespread in the tropics. Although shorter (C_{23}) than the usual carotenoids (C_{40}), bixin also possesses a chain of alternate double-conjugated linkages, which endows it with some special characteristics. In plant seed, bixin is present in the unstable *cis* form, which can be converted into the stable *trans* form with heat extraction and/or the use of organic solvents.[4,5] In the 1980s, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) adjusted the acceptable daily intake (ADI) value for bixin from 1.25 mg/kg bw/day to 0.065 mg/kg bw/day; the latter value is in effect today. Considering that few studies concerning the toxicological safety of these extracts have been performed, in 2002 the Secretariat of JECFA requested studies related to toxicity, specifications, and intake of *annatto* extracts. These studies resulted in ambiguous results regarding bixin/norbixin content, suggesting that consumption of the *annatto* extract has exceeded the JECFA ADI.[6−8] This means that estimates of the bixin/norbixin content of foods were shown to be inaccurate in many cases, which may have led to consumers ingesting more of these extracts than is recommended by the JECFA standards. Therefore, methodologies must be established so that bixin may be used within the current values of ADI.

Curcumin is a naturally occurring compound found in the plant *Curcuma longa*. It has an intense yellow color and is insoluble in water and in ether, is degraded in alkaline solution, and is unstable to light, factors that usually limit its application in foods.[9] For use as a color additive in the food industry, the green curcumin hue can be minimized by combination with an orange hue. It is usual for *annatto* to perform this function, and numerous curcumin/*annatto* blends are commercially available.[10]

Carotenoids are interesting from a pharmaceutical point of view because of their potential usefulness in the treatment of various diseases. Experimental studies clearly indicate that the consumption of saffron positively correlates with a lower risk of certain diseases including metabolic disorders, premenstrual syndrome, depression, insomnia, anxiety, and cardiovascular disease, as well as many types of cancers.[11] With respect to curcumin, the most interesting pharmacological applications are its potential use against cancer, HIV-infections, cystic fibrosis, and as an immunomodulatory agent.[12]

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The conventional production of spices such as curcumin involves hygienic challenges that can pose risks for farmers, producers, and consumers. As a result of these challenges, food quality can be negatively affected. Technologies are currently being developed to address these problems.[13]

Some studies show that the complexation of bixin and curcumin with cyclodextrins (CDs) is a useful and efficient mechanism for protecting these colorants against several environmental factors and harmful agents such as air, ozone, light, and extreme temperatures.[5,12,14,15] To the best of our knowledge, there have been no reports comparing the complexation methodologies of these natural colorants with CDs using differential scanning calorimetry (DSC), thermogravimetry analysis (TGA), or nuclear magnetic resonance (1H-NMR) or evaluating the stability of inclusion complexes for industrial application.

CDs are cyclic oligosaccharides of α-(1,4)-linked-D-glucopyranose units arranged in a ring formation. The most common CDs are α-, β-, and γ-CD, which are formed by six, seven, and eight glucose units, respectively. Of these, β-CD is widely used because its cavity size is suitable for common guests with molecular weights between 200 and 800 g/mol and also because of its ready availability and reasonable price.[16] The hydrophobic internal cavity of CDs renders these compounds capable of forming inclusion complexes with a variety of guest hydrophobic molecules. In this manner, CD can be used to increase the water solubility of the bixin and turmeric oleoresin, which is very hydrophobic and not soluble in water.[15] Microencapsulation protects the oleoresin against destructive changes and converts it into a free-flowing powder.[17] Complexes of curcumin and CDs are thought to be formed by means of the encapsulation by the CD of each of the two phenyl rings at the ends of the curcumin molecule.[18]

Taking into account the importance of natural colorants and their many industrial applications, this study aimed to compare the effectiveness of different methods of formation of complexes of bixin and curcumin with β-CD, to evaluate the stability of the complexes formed and to assess the possibilities for their use in the food industry.

MATERIALS AND METHODS

Samples and Reagents. Bixin (purity 90%) was provided by Christian Hansen (Valinhos, SP, Brazil); curcumin (Sigma) and β-CD (purity 99.5%) were purchased from Sigma Chemical Company (St. Louis, MO, USA). All solvents were of analytical grade.

Spectrophotometric Determination of Colorants. The method of Tang et al.[19] was modified and applied in this study. An aliquot of curcumin stock solution containing $(0.0-4.0) \times 10^{-7}$ mol of curcumin and an aliquot of bixin stock solution containing $(0.0-4.0) \times 10^{-2}$ mol of bixin were added separately to different 10-mL colorimetric tubes, each of which contained 7.5 mL of 1.0×10^{-2} mol/L β-CD and 2.0 mL of trisodium citrate-HCl buffer solution (pH 2.4). The mixtures were diluted to the mark (10 mL) with water, shaken thoroughly, and equilibrated at 20 ± 1 °C for 15 min. The absorbance of the solution was measured at 431 and 488 nm for curcumin and bixin, respectively, against a reagent blank prepared with the same reagent concentration but without curcumin and bixin. This procedure was replicated to obtain three or more absorbance values for each colorant concentration studied. The procedures for the spectrophotometric determination of colorants in the absence of β-CD were similar to those described above except that β-CD was not added to the mixture.

Determination of the Apparent Formation Constant. For the determination of the apparent formation constant, the curcumin and bixin concentrations were held constant at 1.0×10^{-5} mol/L and 1.0×10^{-4} mol/L, respectively. The pH of the system was adjusted to 2.4 by the addition of 2 mL of trisodium citrate-HCl buffer solution (pH 2.4). A β-CD solution was prepared at a concentration of 0.01 mol/L, and 0−5 mL aliquots of this solution were added to the colorants. The mixed solution was diluted to 10 mL with water, shaken thoroughly, and allowed to equilibrate at 20 ± 1 °C for 15 min. The absorbance at 431 nm or at 488 nm for curcumin and bixin, respectively, was read against a reagent blank prepared with the same reagent concentration but without colorants. All measurements were made in duplicate. The apparent formation constant of the inclusion complex was obtained using Benesi−Hildebrand's method (double-reciprocal plot).[20,21] The experimental data were fitted assuming the formation of 1:1 curcumin-β-CD and bixin-β-CD complexes, according to the following reaction:

$$\text{colorant} + \beta\text{-CD} \leftrightarrow \text{colorant} - (\beta\text{-CD})_1 \quad (1)$$

In this reaction, the apparent formation constant (K_f) of the complexes would be given by

$$K_f = [\text{colorant} - \beta\text{-CD}]/[\text{colorant}][\beta\text{-CD}]^1 \quad (2)$$

where [colorant- β-CD], [colorant], and $[\beta\text{-CD}]^1$ are in equilibrium.

The observed relationship between absorbance and concentration of β-CD is given by

$$\frac{1}{A - A_0} = \frac{1}{(A_\infty - A_0)K_f[\beta\text{-CD}]^1} + \frac{1}{A_\infty - A_0} \quad (3)$$

where A is the observed absorbance of the curcumin or bixin solution at each β-CD concentration tested, and A_0 and A_∞ represent the absorbance in the absence of β-CD and when all the curcumin or bixin molecules are complexed, respectively. Therefore, using a plot with values $1/A - A_0$ versus $1/[\beta\text{-CD}]$, the linear relationship that defines the stoichiometric coefficient, and subsequently the value of K_f, is obtained.

Methods of Colorant/β-CD Complex Formation. The formation of β-CD/colorant complexes was carried out using three methods, each of which resulted in inclusion of the target or guest molecule (curcumin or bixin) in the host molecule (β-CD). The methods of preparation of the inclusion complexes are described below.

Coprecipitation. The colorant/β-CD inclusion complex reaction was carried out considering the apparent formation constant (K_f) and the stoichiometry appropriate for each colorant. The amount of colorant required for complex formation when 50 mL of β-CD at a concentration of 0.06 mol/L was used was calculated. The colorants to be complexed were dissolved in a minimum volume of ethanol at 60 °C and then were added dropwise with continuous, intensive stirring to a 250-mL flask with three mouths that contained β-CD aqueous solution at 60 °C. The mixture was refluxed with vigorous agitation at 70 °C for about 4 h. The solution was then stirred for an additional hour at 70 °C to remove ethanol and cooled to room temperature. After stirring for 8 h at ambient temperature, the mixture was stored overnight at 4 °C and then was filtered through a sintered glass filter. The crystalline product that was obtained was dried in a vacuum oven at 50−55 °C.

Kneading. Using a mortar and pestle, 4 mmol of β-CD was kneaded with 4 mL of distilled water/ethanol solution (70:30 v/v) at ambient temperature until homogenization occurred (approximately 5 min). Next, 2 mmol of curcumin or 4 mmol of bixin was added to the paste, and kneading was continued for a further period of 20 min. The resulting paste was dried at room temperature and then powdered.

Simple Mixing. Using a mortar and pestle, 4 mmol of β-CD and 2 mmol of curcumin or 4 mmol of bixin were manually mixed for 20 min.

Characterization of Complexes. *Nuclear Magnetic Resonance.* The complexes were analyzed by NMR spectroscopy (1H-NMR) with a Varian Mercury plus BB model using deuterated water

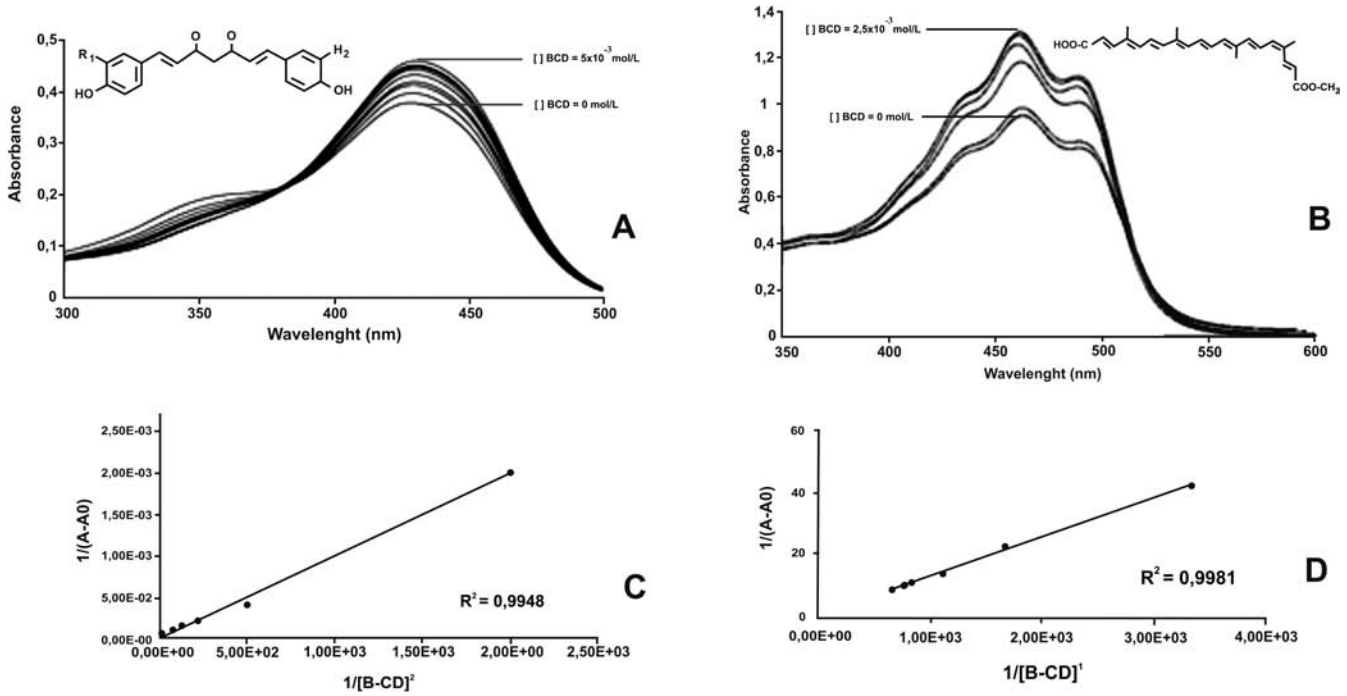

Figure 1. (A) Absorption spectra of curcumin with various concentrations of β-CD. From bottom to top: 5.0×10^{-4}; 1.0×10^{-3}; 1.5×10^{-3}; 2.0×10^{-3}; 2.5×10^{-3}; 3.0×10^{-3}; 4.0×10^{-3}; and 5×10^{-3} mol/L. Curcumin concentration of 1.0×10^{-5} mol/L; pH 2.4; (B) Absorption spectra of bixin with various concentrations of β-CD. From bottom to top: 5.0×10^{-4}; 1.0×10^{-3}; 1.5×10^{-3}; 2.0×10^{-3}; and 2.5×10^{-3} mol L^{-1}. Bixin concentration of 1.0×10^{-4} mol L^{-1}; pH 2.4. (C) Stoichiometric relationship of 1:2 between curcumin and β-CD. (D) Stoichiometric relationship of 1:1 between bixin and β-CD.

as solvent and tetramethylsilane (TMS) as internal reference. The magnetic spectra were obtained at room temperature (22 °C ± 2 °C) with a magnetic field of 300.058 MHz, acquisition time (at) of 2.0 s, pulse (pw) of 45, replications of 128 and a spectral range (sw) of 0−12 ppm.

Differential Scanning Calorimetry (DSC) and Thermogravimetry Analysis (TGA). Aluminum capsules containing approximately 6 mg of sample were analyzed in a Shimadzu calorimeter (model DSC-50 and TGA-50) under a dynamic nitrogen atmosphere (20 mL/min) at a heating rate of 10 °C/min over the temperature range of 20−500 °C for DSC and 20−1000 °C for TGA. DSC calibration was done by the melting point of pattern indium. TGA-50 was calibrated with monohydrated calcium oxalate.

Stability Tests. *Storage Methods.* The colorants curcumin and bixin and the complexes of curcumin-β-CD and bixin-β-CD were stored in 1-g quantities in polyethylene bags, each of which had an area of 100 cm^2. Some of the packages were exposed to natural light, and some were kept in the dark for 53 days at room temperature.[22] Quantitative analyses of the colorants were performed at weekly intervals. To perform these analyses, 0.1 g of powder was placed in a 50-mL Erlenmeyer flask and dispersed in 10 mL of acetone and 10 mL of hexane. This procedure was followed by agitation at 260 rpm for 55 min at 10 °C. Serial dilutions were read in a spectrophotometer and the concentration calculated.[22] The colorant concentration was monitored during the experimental period and was analyzed by linear regression; this helped define the most appropriate mathematical model of reaction kinetics of the colorant for each treatment used in this research.[22,23]

Food Application of Natural Colorants-β-CD Complexes. *Product Preparation.* Because dairy products are often mentioned in the literature as products in which bixin and curcumin are used, yogurt and cheese were chosen to test the suitability of the use of curcumin-β-CD inclusion complexes, and cheese and curd were chosen for testing the application of bixin-β-CD complexes. Yogurt was prepared by adding a cup of yogurt to 3 L of pasteurized milk and maintaining the mixture for 6 h at 40 °C for adequate fermentation. A liter of this yogurt served as a blank, i.e., without colorant; to a second liter, 5 ppm of curcumin was added; and to a third liter, 5 ppm of curcumin-β-CD was added. Minas fresh cheese was prepared using 5 L of pasteurized milk, a tablespoon of enzyme dissolved in 1/4 cup of water, and a tablespoon of yogurt. Three samples in triplicate of cheese were prepared; to the first, colorant was not added; to the second, 20 ppm of curcumin was added; and to the third, 20 ppm of curcumin-β-CD was added. For the manufacture of curd, we used 5 L of milk, cream, and salt. The colorant was added to the samples at the time of cooking. In one sample, colorant was not added; to a second sample, 20 ppm of bixin was added; and to a third, 20 ppm of bixin-β-CD was added.

Determination of Color and Texture. Samples of cheese, curd, and yogurt with the complexes added were analyzed to assess changes in color and texture. The samples were analyzed using a Minolta CR10 colorimeter (Konica Minolta Inc., NJ, USA) with CIE D 65 illuminant at an angle of 8° and a 10° standard CIE observer. The lightness and the red-green and yellow-blue components (L*, a*, and b*) were measured, with three repetitions for each measurement. The polar coordinate Chroma, which indicates color saturation, was estimated as $C^* = (a^{*2} + b^{*2})^{1/2}$. Standard calculation for hue [h* = arc tan(b/a)] was used for the results in the first quadrant [+a*, +b*], while for the second quadrant [+a*, +b*], the calculation h* = 180 + arc tan(b/a) was used. Color was directly measured in the undisturbed surface of the cheese and curd. After the analysis, a 1 cm slice was removed, and color was measured in the inner surface. After homogenization, samples of yogurt were placed in 50 mL plastic cups, and color was measured at the surface.

For the solid products (cheese and curd), texture analyses were done using a TA-XT2i texturometer (Stable Microsystems, Surrey, England). The standard conditions for the tests were as follows: load of 0.05 N, conical metal probe (P/25C) of 25 mm diameter, compression depth of 3 mm, compression speed of 2.0 mm/s, and two compression cycles.

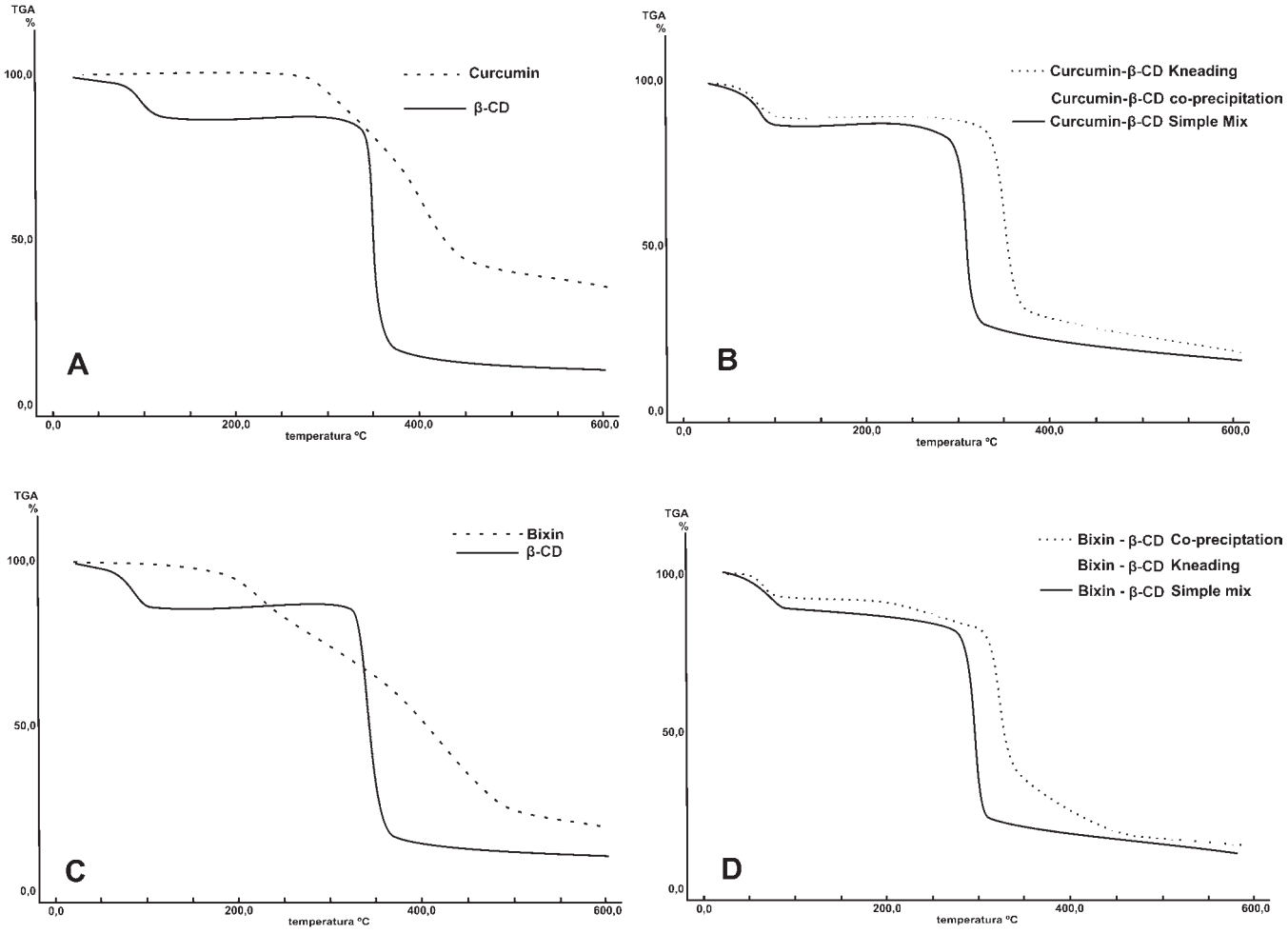

Figure 2. Thermogravimetric analysis of substances: (A) curcumin and β-CD; (B) curcumin-β-CD by different methods; (C) bixin and β-CD; and (D) bixin-β-CD by different methods.

Cheese and curd were cut in pieces of 3 cm diameter and 2 cm height, with a stainless steal cutter. The parameters of texture were obtained from the force−time curves, from which firmness (N), adhesiveness (N·s), springiness, and chewiness (N) were determined.[24] Ten random measurements were made for each sample.

Sensory Analysis. A central local test was carried out in a local supermarket; in this test, routine clients agreed to test the samples and answer some questions. Fifty potential consumers were used to verify the acceptance of the new formulation of cheese and curd with added complex. Forty percent consumed cheese daily, and 23% consumed curd 2 to 3 times per week. A seven-point hedonic scale anchored with verbal terms at the ends and in the middle was used. Purchase intention for the product, the reason for intending to buy/not to buy it, and consumption frequency for each product were asked.

Statistical Analysis. All experiments were carried out using a randomized design. The results were subjected to analysis of variance (ANOVA) with the type of sample as the source of variance and to Tukey means tests (5% significance level), using the software Statistica 7.0/2003 (Statsoft, Inc. Tulsa, OK, USA).

RESULTS AND DISCUSSION

Absorption Spectra of Curcumin and Bixin. The absorption spectra of curcumin at different concentrations of β-CD are shown in Figure 1A. As the β-CD concentration is increased, the absorption maximum of curcumin at 426 nm is slightly red-shifted to 431.5 nm, with a concomitant increase in the absorption intensity. In agreement with the study of Tang et al.,[19] this observation may be explained by partial shielding of the excitable electrons and chromophores in the β-CD cavity and therefore is rationalized as being indicative of complex formation.

Such an effect was not observed for the bixin molecule, because when the β-CD concentration CD was increased, an increase in the intensity of color was also observed, but the absorption maximum of bixin stayed constant, may be due to the absence of aromatic rings in the molecule. However, as with curcumin, when the concentration of bixin was increased, an increase in absorbance at the same wavelength (488 nm), along with increasing variation in β-CD, occurred (Figure 1B); this may be indicative of complex formation.[19]

Determination of the Apparent Formation Constant. Tang et al.[19] have pointed out that, on the basis of the dimensions of the molecules, curcumin is too large to be contained entirely within one β-CD cavity. It is reasonable to consider the formation of a complex involving curcumin and two molecules of β-CD. However, Qi et al.[25] showed that the complexation proportion between curcumin and β-CD was 1:1. To resolve this contradiction and to assist in determining optimal conditions for the complexation of colorant with the host molecule, the present study determined the stoichiometric coefficient of the

Table 1. Thermogravimetric Analysis (TGA) of the Pure Substances and of Products Obtained in the Preparation of Inclusion Complexes

system	complexation technique	decomposition temperature (°C)	stage	TGA (°C)	weight loss (%)
curcumin		270	1	20−175	0.3
			2	175−471	58
			3	471−600	7
β-CD		300	1	24.7−104.0	13
			2	250−387	72
			3	387−600	5
1:2 curcumin-β-CD complex	coprecipitation	280	1	24.7−104	9
			2	250−387	62
			3	387−600	9
	kneading	300	1	24.7−104	10
			2	250−387	62
			3	387−600	9
	simple mixing	220	1	24.7−104	12
			2	250−387	65
			3	387−600	6
bixin		190	1	20−180	3.6
			2	180−480	69.7
			3	480−600	7.3
1:1 bixin-β-CD complex	coprecipitation	194	1	24.7−104	6.5
			2	184−387	61.6
			3	387−600	15.9
	kneading	181	1	24.7−104	10.2
			2	184−387	66.2
			3	387−600	8.8
	simple mixing	190	1	24.7−104	11.3
			2	184−387	69.3
			3	387−600	6.2

complexation, using the method of Benesi−Hildebrand.[20,21] When values of $1/(A - A_0)$ (see eq 3, Materials and Methods) were plotted as a function of $1/[\beta\text{-CD}]$, a nonlinear relationship was observed that reflected a complex stoichiometry ratio of 1:2 (Figure 1C). The value obtained for the apparent formation constant (K_f) of this complex was 4×10^4 mol^{-2}L^2.

The complexation of bixin with β-CD, to our knowledge, has been studied only by Lyng et al.;[5] these authors did not establish the stoichiometric ratio of the complex. Thus, in this study we evaluated the possible stoichiometric ratios 1:1 and 1:2; a 1:1 ratio is suggested by the fact that bixin showed a linear relationship with $R^2 = 0.9981$ (Figure 1D). The apparent formation constant (K_f) for the complex of bixin with β-CD was 3.34×10^2 mol^{-1}L.

On the basis of these results, it is supposed that in the inclusion complex each of the benzene rings of curcumin was contained within one β-CD cavity by means of van der Waals forces and hydrophobic interactions. Furthermore, curcumin is a molecule with electron donor groups able to form hydrogen bonds within the β-CD cavity, which may make the 1:2 complex more stable. Thus, it is likely that bixin, which lacks benzene rings at its ends, forms an inclusion complex with β-CD that is less stable than that formed between β-CD and curcumin. This is supported by the relative observed values of K_f, which are lower for the bixin-β-CD complex than for the curcumin-β-CD complex.[19]

Characterization of Complexes by DSC and TGA. Curcumin is a molecule of low hydrophilicity; therefore, TGA of curcumin-β-CD complexes did not show a stage of water loss at 25−104 °C similar to that exhibited by pure β-CD. At 246 °C, the complex began to lose weight continuously up to 450 °C; this is shown by the data presented in Figure 2A and Table 1. One factor that indicates the formation of the inclusion complex is the change in decomposition point.[26] As can be seen in Figure 2B, the curcumin-β-CD complex prepared by the coprecipitation method showed the greatest change in decomposition point. The curcumin-β-CD complex prepared by the kneading method had a decomposition point similar to that of pure β-CD. The simple mixing yielded a complex that was thermodynamically less stable than the other two, with weight loss that began to occur 60 °C before the mass loss that occurred with the coprecipitation method. For bixin-β-CD complexes, significant weight loss began to occur near 190 °C, and a downward inclination was observed up to 450 °C (Figure 2C). Figure 2C and D and Table 1 show that the curves for pure β-CD and for the bixin-β-CD complex prepared by the kneading method essentially overlapped, indicating the presence of β-CD in the free form. For the bixin-β-CD complex prepared by the coprecipitation method, near the decomposition temperature of β-CD (300 °C), there was an inclination up to 450 °C, indicating a slow decomposition stage, i.e., interference of the colorant with the degradation of the host molecule. The bixin-β-CD complex prepared by simple mixing was less stable than the other two complexes. Similar results have not previously been reported in the literature.

An endothermic peak was observed at 175 °C in the curcumin DSC thermogram (Figure 3A); this peak represents thermal

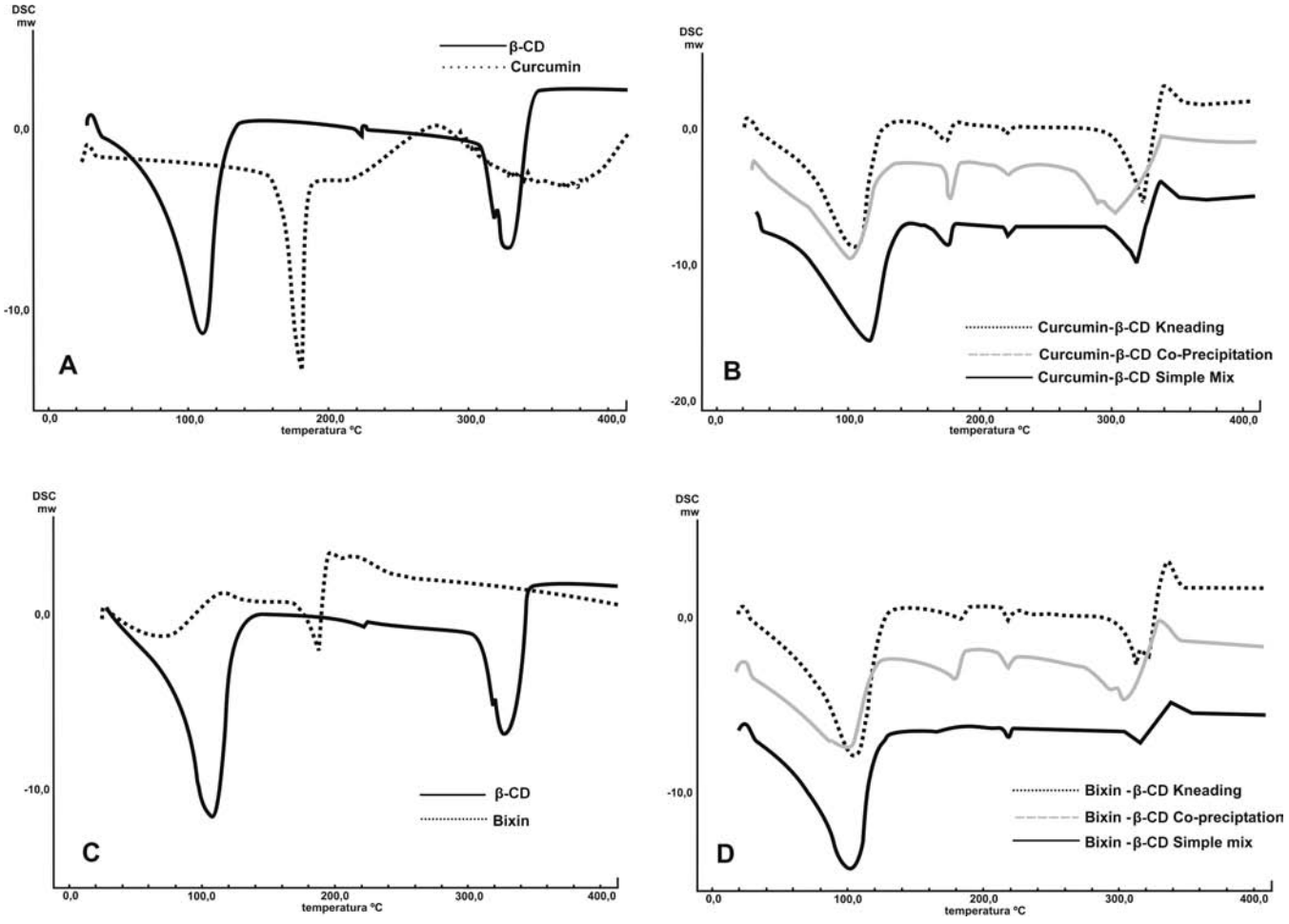

Figure 3. DSC thermogram of substances: (A) curcumin and β-CD; (B) curcumin-β-CD by different methods; (C) bixin and β-CD; and (D) bixin-β-CD by different methods.

Table 2. DSC Thermogram Results for the Pure Substances and for Products Obtained in the Preparation of Inclusion Complexes[a]

	water evaporation (°C)	ΔH (kJ/mol)	colorant degradation (°C)	ΔH (kJ/mol)	β-CD degradation (°C)	ΔH (kJ/mol)
β-CD	104.7	439.2			326	177
curcumin			176	123.8		
curcumin-β-CD by KM	106.6	409.1	176.6	18.4	325.4	164.2
curcumin-β-CD by CPM	103.2	340.4	180.4	14.2	307.5	322
curcumin-β-CD by SMM	116.7	368.7	177.5	15.7	322	79.2
bixin			185.5	58.6		
bixin-β-CD by KM	107.3	395.5	184.2	7	314	92
bixin-β-CD by CPM	103	263	182.8	22	298.5	126.4
bixin-β-CD by SMM	101	390.8	165.9	1.3	315.5	23.9

[a] KM, kneading method; CPM, co-preciptation method; SMM, simple mixing method.

degradation of the substance and indicates the absence of a melting point for curcumin. According to Brady and Humiston,[26] the greater the variation in temperature and enthalpy of the system, the greater the possibility is of complex formation due to changes in physical characteristics of the components. Figure 3B and Table 2 show that, for the complex prepared by the coprecipitation method, the change in water evaporation enthalpy represented by the endothermic peak was lower in terms of energy, indicating a better interaction between curcumin and β-CD. This complex also promoted greater thermal protection of curcumin, causing degradation from 176 to 180.45 °C, and its enthalpy change was also the most significant. The DSC values obtained from samples prepared by kneading and simple mixing were similar to those of pure β-CD, indicating that β-CD is present in free form in these samples.

In the DSC thermogram shown in Figure 3C, the curves for β-CD and for pure bixin are shown. The colorant showed an endothermic peak at 68 °C resulting from the loss of water and

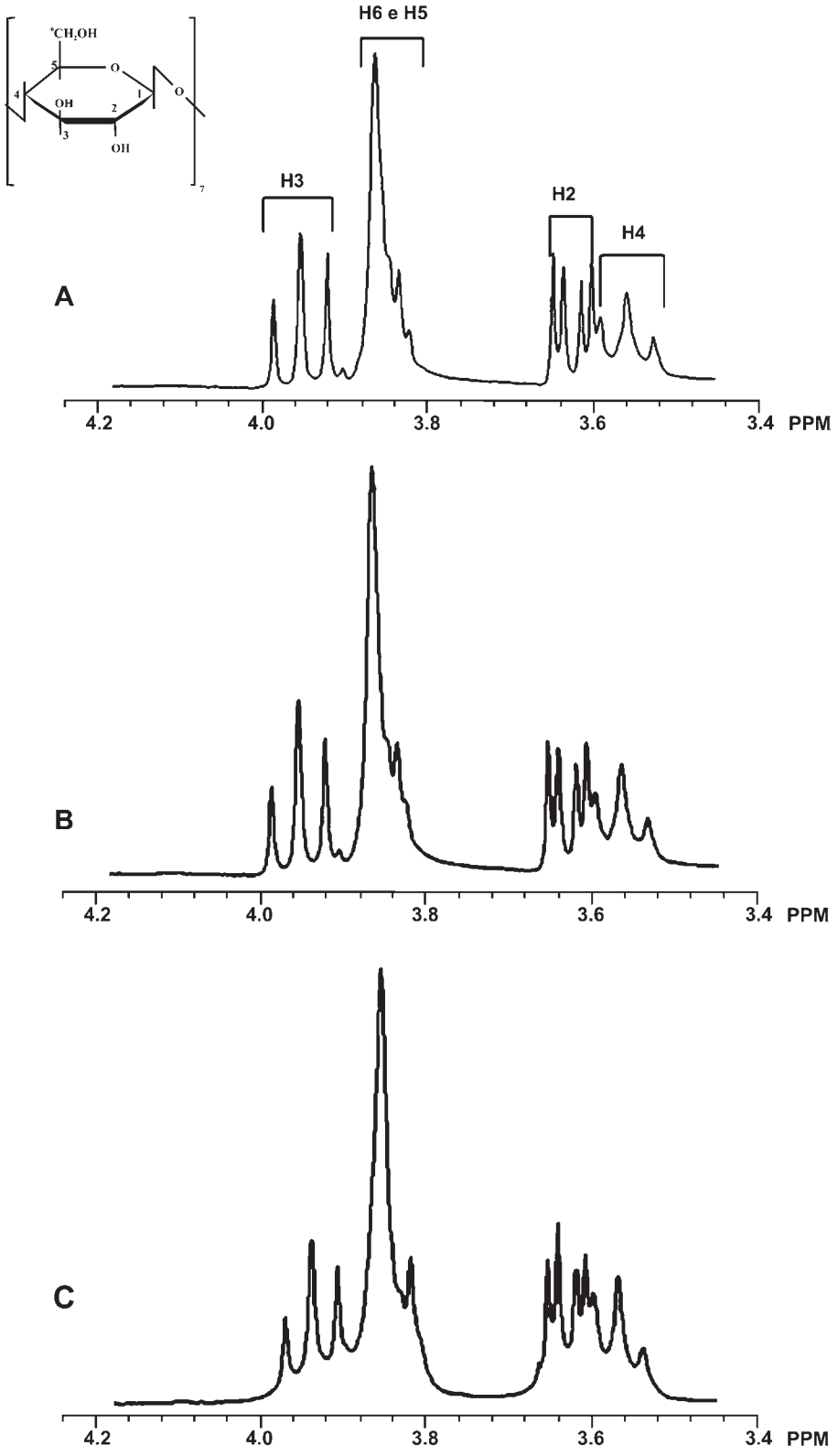

Figure 4. H^1-NMR spectrum for (A) β-CD. Top and left: the drawing explains the numbering of the hydrogens positioned in the molecule. (B) Curcumin-β-CD complex prepared by the coprecipitation method. (C) Bixin-β-CD complex prepared by the coprecipitation method.

another at 185 °C that represents its thermal degradation. The data in Figure 3D and Table 2 show that the coprecipitation method resulted in a complex with water evaporation enthalpy that was energetically inferior to that of the complexes obtained by the other methods, indicating a better interaction between bixin and β-CD in the coprecipitation complex. The bixin-β-CD complex obtained by the coprecipitation method also showed greater enthalpy variation compared to pure β-CD than the

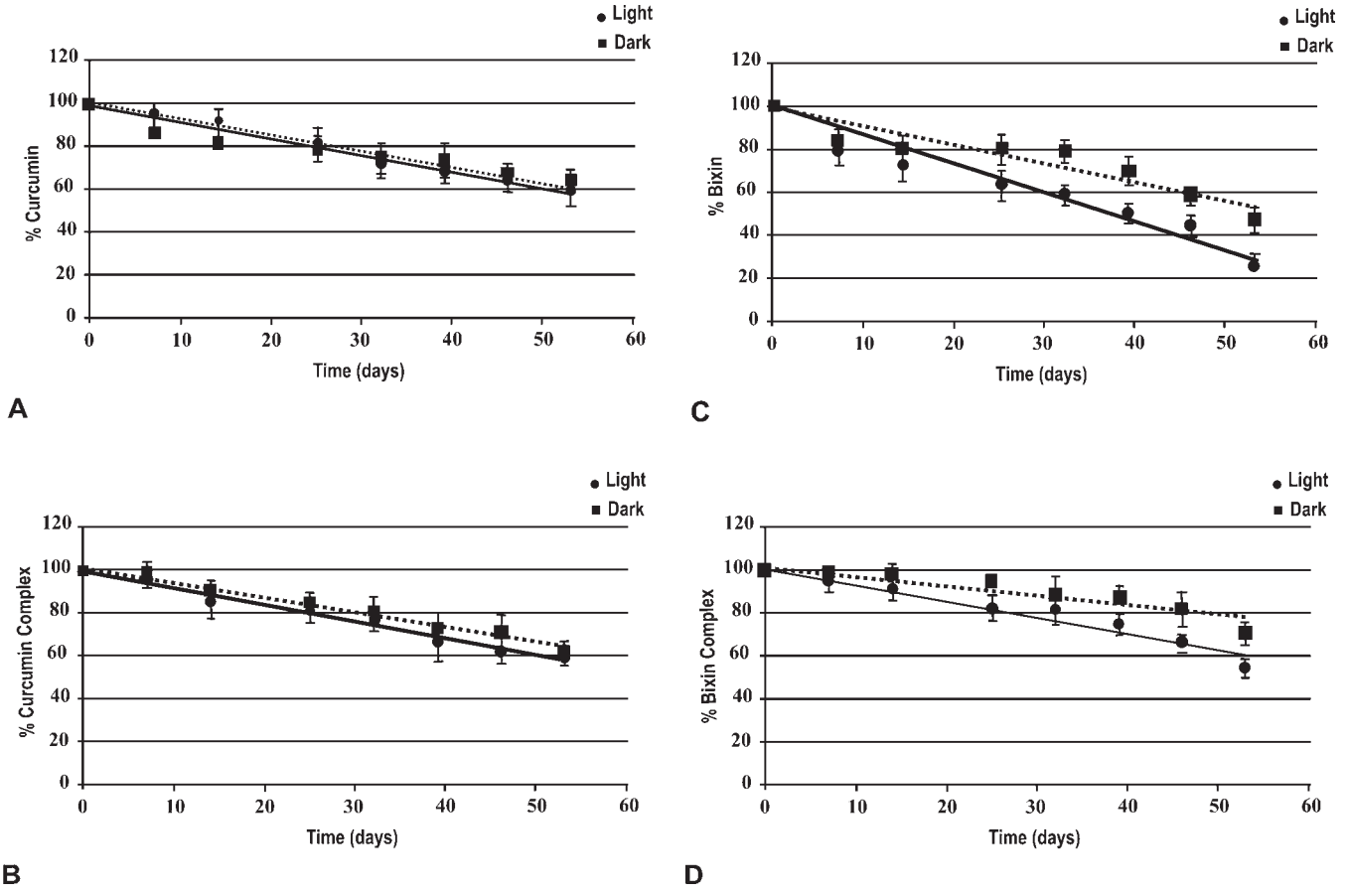

Figure 5. Remaining percentage of substances during storage in the presence and absence of light: (A) curcumin; (B) curcumin-β-CD complex; (C) bixin; and (D) bixin-β-CD complex.

complexes obtained by other methods, confirming the ability of this method to produce physical alteration of the molecule.

Spectrophotometric measurement of the supernatant acquired after obtaining complexes by the coprecipitation method showed that 13.7% of curcumin and 22% of bixin remained in the liquid supernatant.

Characterization of Complexes by NMR. CD rings are amphipathic, with the wider rim displaying the 6-OH group on its flexible arm. Thus, the hydrophilic groups are on the outside of the molecular torus, whereas the hydrophobic inner surface is lined with the ether-like anomeric oxygen atoms and the C3-H and C5-H hydrogen atoms.[15] Figure 4A shows the hydrogens of interest in NMR analysis for the possible identification of complex formation. When there is complex formation, i.e., when the guest molecule enters the CD cavity, the signals of H3, H5, and H6 change, indicating complex formation. However, it was not possible to obtain significant results for analysis by NMR for either curcumin (Figure 4B) or bixin (Figure 4C) due to the fact that the substances are practically insoluble in water. Because changes in the peaks by contact of curcumin and bixin with β-CD were small or none, it was not possible to affirm the formation of complexes by this method. According to Wimmer et al.,[27] aliphatic guest complexes or complexes with low association constants often display insignificant chemical shifts; this could explain the small chemical shifts in the β-CD peaks for these complexes.

Stability Tests. For curcumin, a decay of up to 40% in the intensity of color occurred during the test period (Figure 5A). However, the loss of color was not primarily due to exposure to light because the colorant stored in the dark showed a loss of color intensity similar to that of the compound stored in the presence of natural light. Furthermore, complexation of the dye with β-CD does not appear to improve preservation against light. Comparison of Figure 5A with Figure 5B shows that, even when curcumin is complexed with β-CD, approximately 40% decay in color intensity occurs both with storage under light and in the dark, similar to the loss that occurs without complexation.

Bixin stored under light showed a decay in color intensity of about 75%, while storage in the dark resulted in a decrease of approximately 50%; thus, light appears to interfere significantly with the integrity of this colorant (Figure 5C). Complexation of bixin with β-CD conferred some protection against light; the decay of color was lower than that of the colorant without complexation (Figure 5D). Measurement of bixin color intensity after storage in the dark showed that the difference between complex and colorant without complexation was slightly smaller than that which occurred in the presence of light, again suggesting a protective action by β-CD.

Food Application of Complexes of Natural Colorant-β-CD. *Determination of Color and Texture.* There were no significant differences in the firmness, adhesiveness, springiness, or chewiness of products containing added coloring and those containing bixin-β-CD or curcumin-β-CD complexes, indicating that complexation did not alter the texture profile of the cheese (Table 3) or curd (Table 5). In all cases, the addition of colorants and complexes reduced the brightness similarly so that cheese

Table 3. Parameters of Texture and the Color of the Cheese Crust[a]

		cheese with curcumin	cheese with curcumin-β-CD	cheese without colorants
crust	(L*)	86.0 ± 1 b	82.1 ± 1 b	89.1 ± 1 a
	(h*)	95.0 ± 1.1 a	90.5 ± 1.3 b	90.3 ± 1.1 b
	(C*)	17.1 ± 2.3 b	43.0 ± 1.8 a	13.0 ± 1.7 c
firmness (N)		22.1 ± 1.8 a	23.0 ± 2.8 a	21.1 ± 1.2 a
adhesiveness (N·s)		−0.1 ± 0.08 a	−0.07 ± 0.06 a	−0.05 ± 0.6 a
springiness		0.9 ± 0.03 a	0.9 ± 0.05 a	0.8 ± 0.08 a
chewiness (N)		16.9 ± 1.3 a	18.2 ± 2.9 a	18.0 ± 1 a

[a] The same letters in the same line indicate that there was no significant difference between the samples ($p \leq 0.05$). L* = lightness (0 = black; 100 = white); C* = chroma; h* = hue.

Table 4. Parameters of Color for Yogurt[a]

	yogurt with curcumin	yogurt with curcumin-β-CD	yogurt without colorant
(L*)	79.6 ± 0.1 b	79.7 ± 0.1 b	80.8 ± 0.1 a
(h*)	106.9 ± 0.2 b	106.3 ± 0.2 b	114.0 ± 0.3 a
(C*)	9.6 ± 0.3 b	25.2 ± 0.1 a	7.9 ± 0.2 c

[a] The same letters in the same line indicate that there was no significant difference between the samples ($p \leq 0.05$). L* = lightness (0 = black; 100 = white); C* = chroma; h* = hue.

Table 5. Parameters of Texture and the Color of the Curd Crust[a]

		curd with bixin	curd with bixin-β-CD	curd without colorant	cheddar
crust	(L*)	66.1 ± 0.3 d	71.0 ± 0.3 b	81.1 ± 0.5 a	69.0 ± 0.4 c
	(h*)	77.5 ± 0.1 c	76.0 ± 0.0 b	96.7 ± 0.2 a	76.0 ± 0.1 b
	(C*)	36.9 ± 0.1 c	41.2 ± 0.2 b	17.4 ± 0.2 d	45.3 ± 0.3 a
firmness (N)		15.0 ± 0.9 a	14.0 ± 0.5 a	14.0 ± 0.9 a	
adhesiveness (N·s)		−0.1 ± 0.0 a	−0.1 ± 0.0 a	−0.1 ± 0.0 a	
springiness		0.7 ± 0.0 a	0.8 ± 0.0 a	0.8 ± 0.0 a	
chewiness (N)		7.0 ± 0.4 a	7.5 ± 0.4 a	7.7 ± 0.7 a	

[a] The same letters in the same line indicate that there was no significant difference between the samples ($p \leq 0.05$). L* = lightness (0 = black; 100 = white); C* = chroma; h* = hue.

(Table 3), yogurt (Table 4), and curd (Table 5) without the addition of colorants or complexes showed lighter color.

For cheese and yogurt to which curcumin or curcumin-β-CD was added, the change in color saturation (chroma) was more significant than in the hue. The cheeses showed a yellow hue (h* of 90.3−95.0) and the yogurt a slightly greenish-yellow hue (h* of 106.3−114.0), independent of the addition of colorant or the complex.

For products without colorant or the complex, lower chroma was observed than for products with curcumin. Products with the complex showed color approximately 2.5 times higher, confirming the greater capacity of the complex to provide color to the product (Tables 3 and 4).

The addition of bixin and bixin-β-CD influenced both the chroma and the hue of curd, but in this case, the behavior of the colorant and complex was quite similar. Product without colorant showed a slightly greenish-yellow hue (h* of 96.7), while those to which bixin or the bixin complex had been added were characterized by a reddish-yellow hue (h* from 76.0 to 77.5). The chroma of the product with bixin or bixin-β-CD added was approximately 2-fold higher than that of cheese without added colorant and complex. For purposes of comparison, commercial cheddar cheese was also analyzed; it was found that the addition of the complex was important to obtain a product with brightness, hue, and chroma similar to those in the market. There were no significant differences in texture between the curd with added dye or complex (Table 5).

The inner color of the cheeses and curds were similar to the surface ones. The main difference observed was for the curd with bixin as the interior presented darker color (L* of 72), similar to the surface color of the curd with bixin-β-CD.

Sensory Analysis. Both the fresh cheese with added curcumin-β-CD and the curd with added bixin-β-CD were well accepted, receiving average scores of 6.7 and 5.7, respectively, on a scale of 7 points. When panelists were requested to mention the least-liked attributes of the products, a few remarks were offered, but there was no consensus. With respect to attributes that they liked best, the panelists referred to the taste of the product (60% for cheese and 78% for curd) and to its color (43% for cheese and 25% for curd).

The results obtained in this research confirm that, for curcumin, the best method of complexation was coprecipitation. The value of the apparent formation constant, K_f, was significant, and a stoichiometric relationship of 1:2 between curcumin and β-CD was established. For bixin, the most effective complexation method with respect to thermal protection and complex formation was also coprecipitation, and a stoichiometric relationship of 1:1 between bixin and β-CD was established. Consistent with the idea that the interaction of the bixin molecule with the internal cavity of CD is less intense than that of curcumin, the value of K_f

for the bixin-β-CD complex was lower than that obtained for the curcumin complex; however, the solubilization capacity in aqueous media increased the possibility of using bixin in low-fat foods, expanding the field of industrial application. For curcumin, it was observed that the light is not the only factor that influences stabilization of the colorant. However, for bixin, the presence of light led to its decomposition, and complexation with β-CD led to significant improvement in maintaining its color intensity. Instrumental color testing proved that curcumin-β-CD and bixin-β-CD have a greater ability to provide color than the pure colorants. Instrumental analysis of texture showed that the use of the complexes in dairy products do not alter their initial characteristics. The application of the complexes in food had good sensorial acceptability.

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ABBREVIATIONS USED

JECFA, Joint FAO/WHO Expert Committee on Food Additives; ADI, acceptable daily intake; CDs, cyclodextrins; β-CD, β-cyclodextrin; DSC, differential scanning calorimetry; TGA, thermogravimetry analysis; NMR-H, nuclear magnetic resonance; ΔH, enthalpy variation.

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