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Curcumin–β-cyclodextrin inclusion complex: Stability, solubility, characterisation by FT-IR, FT-Raman, X-ray diffraction and photoacoustic spectroscopy, and food application

Camila Sampaio Mangolim [a], Cristiane Moriwaki [b], Ana Claudia Nogueira [c], Francielle Sato [c], Mauro Luciano Baesso [c], Antônio Medina Neto [c], Graciette Matioli [a,b,*]

[a] Postgraduate Program in Food Science, State University of Maringá (UEM), Av. Colombo 5790, 87020-900 Maringá, PR, Brazil
[b] Department of Pharmacy, State University of Maringá (UEM), Av. Colombo 5790, 87020-900 Maringá, PR, Brazil
[c] Department of Physics, State University of Maringá (UEM), Av. Colombo 5790, 87020-900 Maringá, PR, Brazil

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ABSTRACT

Curcumin was complexed with β-CD using co-precipitation, freeze-drying and solvent evaporation methods. Co-precipitation enabled complex formation, as indicated by the FT-IR and FT-Raman techniques via the shifts in the peaks that were assigned to the aromatic rings of curcumin. In addition, photoacoustic spectroscopy and X-ray diffraction, with the disappearance of the band related to aromatic rings, by Gaussian fitting, and modifications in the spectral lines, respectively, also suggested complex formation. The possible complexation had an efficiency of 74% and increased the solubility of the pure colourant 31-fold. Curcumin–β-CD complex exhibited a sunlight stability 18% higher than the pure colourant. This material was stable to pH variations and storage at −15 and 4 °C. With an isothermal heating at 100 and 150 °C for 2 h, the material exhibited a colour retention of approximately 99%. The application of curcumin–β-CD complex in vanilla ice creams intensified the colour of the products and produced a great sensorial acceptance.

1. Introduction

The use of natural dyes is important in the consumer acceptance of foods. Curcumin, a hydrophobic yellow-orange polyphenol derived from the rhizome of the herb *Curcuma longa*, is an important natural colourant, that is used in food and pharmaceutical preparations (Anand, Kunnumakkara, Newman, & Aggarwal, 2007; Wang, Lu, Lv, & Bie, 2009; Zhang, Zhang, Yu, Bao, Sun & Lu, 2013). In the food industry, curcumin is added as a stabiliser in jellies or is used as a natural colourant, as a substitute for artificial colourants in cheeses, pickles, mustards, cereals, soups, ice creams and yogurts (Paramera, Konteles, & Karathanos, 2011a). Curcumin is also a very interesting pharmacological compound due to its innumerous pharmacological applications, including anti-inflammation, anti-human immunodeficiency virus, anti-microbial, anti-oxidant, anti-parasitic, anti-mutagenic and anti-cancer (Mohan, Sreelakshmi, Muraleedharan, & Joseph, 2012; Singh, Tonnesen, Vogensen, Loftsson, & Másson, 2010; Yallapu, Jaggi, & Chauhan, 2010). It is considered safe for human use, even in high doses (Anand et al., 2007; Singh et al., 2010).

The applications of curcumin are limited due to its low water solubility and sensitivity to alkaline conditions, thermal treatment, light, metallic ions, enzymes, oxygen and ascorbic acid. Additionally, curcumin is poorly absorbed in the gut, independent of the route of administration, which limits its bioavailability (Paramera, Konteles, & Karathanos, 2011b). These factors usually restrict the application of curcumin in the food industry and in pharmaceutical formulations. Thus, an improvement in the stability and solubility of curcumin is necessary. Microencapsulation is a technique that is commonly used to overcome these disadvantages (López-Tobar, Blanch, Castillo, & Sanchez-Cortes, 2012; Paramera et al., 2011a, 2011b; Wang et al., 2009).

The encapsulation of curcumin has been described in the literature using different materials. Among others, gelatin (Wang et al., 2009), cyclodextrins (CDs) (Mohan et al., 2012; Szente, Mikuni, Hashimoto, & Szejtli, 1998; Tonnesen, Másson, & Loftsson, 2002), cationic micelles (Leung, Colangelo, & Kee, 2008), liposomes (Li, Braiteh, & Kurzrock, 2005), yeast cells (Paramera et al., 2011a) and modified starch (Paramera et al., 2011b; Yu & Huang, 2010) are some of the most used encapsulating agents. In this context, CDs offer advantages over other materials because they possess a hydrophobic cavity in which a wide variety of lipophilic guest molecules can be hosted. They are cyclic oligosaccharides with six, seven or eight glucose units linked by α-(1,4)-glucosidic bonds,

* Corresponding author. Tel.: +55 44 3011 3868; fax: +55 44 3011 4119.
E-mail address: gmatioli@uem.br (G. Matioli).

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named, respectively, α-, β- and γ-CD (Gomes, Petito, Costa, Falcão, & Araújo, 2014). CDs are non-toxic ingredients and, of the three CDs, β-CD is the most widely used because its cavity fits common guests with molecular weights between 200 and 800 g/mol and also because of its ready availability and reasonable price (Szente & Szejtli, 2004). According to Marcolino, Zanin, Durrant, Benassi, and Matioli (2011) and Tang, Ma, Wang, and Zhang (2002), curcumin forms inclusion complexes with β-CD in a 2:1 (host:guest) molar ratio, in which a CD encapsulates one of the two phenyl rings of curcumin.

The inclusion complexes that are formed between CD and its guest need to be characterised using analytical techniques. In many papers, Fourier transform infrared spectroscopy (FT-IR) was used to study complex formation between curcumin and β-CD (Tang et al., 2002) or hydroxypropyl-β-CD (Mohan et al., 2012; Yallapu et al., 2010). Mohan et al. (2012) and Yallapu et al. (2010) obtained vague details as evidence of complexation. Tang et al. (2002) presented reasonable evidence for the formation of inclusion complexes. Fourier transform Raman scattering infrared spectroscopy (FT-Raman) is considered to be an excellent tool for studying inclusion complex formation. Although Raman spectral studies of curcumin and of curcumin–CD complexes for characterisation have been reported (López-Tobar et al., 2012; Mohan et al., 2012), this technique was not successfully used to characterise curcumin–β-CD inclusion complexes. Photoacoustic spectroscopy is an innovative and low-cost technique that provides many advantages above others that are commonly used (i.e., it is non-destructive and requires little sample preparation). This technique can also be valuable for investigation of complex formation between β-CD and guest molecules (Dias, Berbicz, Pedrochi, Baesso, & Matioli, 2010; Dóka, Prágai, Bicanic, Kulcsár, & Ajtony, 2013).

The aim of this work was to compare different methods of curcumin complexation with β-CD and to evaluate the formation of the complexes using the FT-IR, FT-Raman, X-ray diffraction and photoacoustic spectroscopy techniques. The solubility and stability against light, pH, storage and thermal treatment of the formed complexes were evaluated. Additionally, the food application of the complexes in vanilla ice cream was investigated.

2. Materials and methods

2.1. Materials

Curcumin and β-CD were purchased from Sigma Chemical Company (St. Louis, MO, USA). All solvents were of analytical grade.

2.2. Methods for complex preparation

The inclusion complexes of curcumin and β-CD were prepared in the molar ratio of 1:2 using co-precipitation, freeze-drying and solvent evaporation techniques.

2.2.1. Co-precipitation

The method described by Marcolino et al. (2011) was used with a few modifications. An aqueous solution of β-CD with a concentration of 0.06 mol/L was stirred at 60 °C. Curcumin, 1.9 g, was dissolved in ethanol at 60 °C and added to the solution. The mixture was refluxed with vigorous agitation at 70 °C for 4 h. The ethanol was removed using a rotary evaporator. The solution was cooled to 25 °C, stirred for 8 h and stored overnight at 4 °C. Afterwards, it was filtered, and the obtained crystalline product was dried at around 50–55 °C and stored for further measurements.

2.2.2. Freeze-drying

The method described by Mohan et al. (2012) was used with a few adaptations. β-CD was dissolved in 30 ml of water in a 250 ml stoppered conical flask and stirred until a clear solution was obtained. To this solution, 210 mg of curcumin were added, which had been previously diluted in ethanol. Another solution was prepared in a similar manner without the use of ethanol. The reaction mixtures were stirred in an incubator shaker at 180 rpm for 7 days at 37 °C. Afterwards, the mixtures were filtered through a 0.45 μm filter, and the clear solutions were freeze-dried to obtain solid complexes, which were stocked for characterisation.

2.2.3. Solvent evaporation

The method described by Yallapu et al. (2010) was used for solvent evaporation. β-CD was dissolved in 8 ml of water in a glass vial with a magnetic stirrer bar. While the solution was stirred, 16.9 mg of curcumin dissolved in 500 μl of acetone was added. The solution was stirred overnight and centrifuged at 134 × *g* for 5 min. The supernatant, which contained the complex, was recovered by freeze-drying and was stored for characterisation.

2.3. Curcumin quantification

To determine the curcumin content in the pure curcumin samples, 10 mg of the sample were diluted in 25 ml of ethanol and then filtered. The absorbance of the solution was determined at 430 nm using a UV–Vis spectrophotometer (model Genesys 20, Thermo Spectronic, USA).

To determine the curcumin content in the complexes, 10 mg of curcumin–β-CD complex were dissolved in 10 ml of ethanol, filtered and the absorbance was determined at 430 nm. For each measurement, the baseline was established using blank ethanol as a reference. The complexation efficiency (CE) was determined in accordance with Paramera et al. (2011a) and Wang et al. (2009). CE (%) is defined as the ratio between the amount of complexed curcumin and the total amount added initially:

$$\mathrm{CE}\ (\%) = \frac{C_E}{C_T} \times 100$$

C_E refers to the mass of complexed curcumin and C_T to the total mass of curcumin added initially.

2.4. Inclusion complex characterisation

The FT-IR spectra of curcumin, β-CD, simple mixtures and complexes were obtained using an infrared Fourier transform spectrometer (model Vertex 70v, Bruker, Germany). The spectral range was 400–4000 cm^{-1} with 128 scans and a resolution of 2 cm^{-1}. The samples were diluted in KBr powder and pellets were made to perform the measurements.

The Raman spectra of curcumin, β-CD, simple mixture and complexes were obtained using an infrared Fourier transform spectrometer (model Vertex 70v with Ram II module, Bruker, Germany) equipped with a liquid nitrogen cooled Germanium detector. A Nd:YAG laser was used for excitation at 1064 nm with 5 up to 200 mV. All of the spectra were an average of 500 scans with a 4 cm^{-1} resolution.

The photoacoustic measurements of curcumin, β-CD, simple mixtures and complexes were performed using a custom-built experimental setup, the same as the one used by Dias et al. (2010). All of the spectra were obtained at a modulation frequency of 21 Hz, recorded between 200 and 800 nm and normalised with a carbon black spectrum. The spectra were analysed by Gaussian deconvolution.

The X-ray diffractograms of curcumin, β-CD, simple mixtures and curcumin–β-CD complex from co-precipitation were obtained

using a X-ray diffractometer (model LabX XRD-6000, Shimadzu, Japan), and the samples were investigated in the 2θ range of 2°–60°.

2.5. Solubility assay

Samples of 4.5 mg of pure curcumin and of 32 mg curcumin–β-CD complexes with the molar ratio of 1:2 were placed in 10 ml test tubes. In the tubes, 8 ml of deionised water were added and stirred for 1 min. The contents of the tubes were centrifuged, and an aliquot was taken for spectrophotometric analysis at 430 nm.

2.6. Stability studies

2.6.1. Natural light stability

The photochemical stability of the curcumin–β-CD complex and pure curcumin was assessed using the procedure described by Paramera et al. (2011b) with a minor modification. For 1 month, 160 mg of pure curcumin and 1.14 g of the curcumin–β-CD complex with a molar ratio of 1:2 were exposed to sunlight in enclosed glass Petri dishes. After exposure for 5, 10, 15, 20, 25 and 30 days, samples were collected, and the percentage of curcumin retention was measured using a spectrophotometer, as described in Section 2.3.

2.6.2. pH stability

Pure and complexed curcumin, 7 and 2.4 ppm of colourant, respectively, were diluted in a water:ethanol solution with a 70:30 (v/v) proportion. The solutions, 4 ml, were adjusted to pH values in the range of 1–9 using buffer solutions and the absorbance was determined at 430 nm.

2.6.3. Storage stability

Pure and complexed curcumin were placed in amber glass bottles and stored for 90 days at −15, 4 and 25 °C. An aliquot of each sample was collected every 15 days and the curcumin content was determined by spectrophotometric analysis at 430 nm as described in Section 2.3.

2.6.4. Thermal stability

The thermal stability of pure and complexed curcumin was assessed in accordance with the method described by Paramera et al. (2011b), with a minor modification. Isothermal heating was conducted under oxidative conditions. During the process, 10 mg of the samples were heated at 100, 150 and 200 °C for 30, 60 and 120 min. After thermal treatment, the samples were diluted in 20 ml of ethanol and the curcumin content was measured as described in Section 2.3.

2.7. Food application

Curcumin is commonly used in dairy products. Thus, in this work, vanilla ice cream was chosen to test the use of the curcumin–β-CD inclusion complex. Three formulations were prepared (A, B and C). Visual tests and colourimetric measurements were performed to determine the quantity of pure colourant that was necessary to obtain the commercial vanilla ice cream colour. The required quantity of the pure colourant in 1 L of milk was 250 ppm (formulation A). From this determination, a second formulation was made that contained the complex with 250 ppm of colourant (formulation B) and another formulation was made that contained the quantity of complex that was equivalent to the colour of formulation A, i.e., 300 ppm (formulation C).

The ice cream was prepared by adding 60.0 g of milk fat, 250.0 g of sugar, 30.0 g of a powder mixture, which contained maltodextrin and vanilla flavouring, and the pure or complexed colourant into 400 ml of ultra high temperature (UHT) whole milk at 40 °C. For the complete solubilisation of the formulation that contained the pure colourant, vigorous stirring with a mixer was needed. The formulations were placed in the ice cream machine (model MSP-4, Eletro Real Frio, Brazil) and 600 ml of UHT whole milk were added. After 10 min of homogenisation, 10.0 g of an emulsifier and 10.0 g of a stabiliser were added. The preparations were ready after 20 min of additional homogenisation and were stored at −15 °C for further analysis.

2.7.1. Sensory evaluation

A sensory evaluation of the three formulations of vanilla ice cream was conducted on a laboratory scale, in individual booths under white light and with 80 untrained panellists. A hedonic scale of 9 points (with 1 as "dislike extremely" and 9 as "like extremely") was used to evaluate the acceptability of the colour, texture, taste and flavour of the products; while a scale of 3 points (with 1 as "certainly would not buy" and 3 as "certainly would buy") was used for a buying analysis. The samples were placed in plastic glasses that were coded with 3 digit random numbers and were presented randomly to the panellists (Stone & Sidel, 2004, chap. 7).

2.7.2. Colour determination

The samples were analysed for variations in colour in accordance with Marcolino et al. (2011). The measurements were made using a colorimeter (model CR-400, Konica Minolta Sensing Inc., Japan) with an 8 mm aperture and diffuse illumination (D65 illuminant, 0° viewing angle). Readings were reported in the CIE Lab system (1931).

The lightness and the red–green and yellow–blue components (L^*, a^*, and b^*) were determined with three repetitions. The L^* axis represents the darkness and lightness coordinate, with values ranging from 0 (perfect black) to 100 (perfect white). The a^* axis symbolises chromaticity coordinates, in which green signifies negative and red positive coordinates. The b^* axis also symbolises chromaticity coordinates, in which yellow signifies positive and blue negative coordinates (Tung, Goldstein, Jang, & Hittelman, 2002). Colour was directly measured in the surface of the ice creams and in the inner surface, when slice of about 1 cm was removed.

Chroma measures colour saturation or intensity, while the hue angle is used to discriminate among subtle visual colour differences (Perkins-Veazie, Collins, Pair, & Roberts, 2001). The chroma and hue were calculated with the following equations (Arias, Lee, Logendra, & Janes, 2000):

$$\text{Chroma} = \sqrt{a^{*2} + b^{*2}}.$$

Hue = $180 + \tan^{-1}$ (b^*/a^*) for results in the second quadrant [$-a^*$; $+b^*$].

2.8. Statistical analysis

Data were evaluated using analysis of variance (ANOVA), and means were compared with Tukey test ($p < 0.05$) using the software Statistica 8.0/2008 (Stat Soft, Inc., Tulsa, USA).

3. Results and discussion

3.1. Inclusion complex characterisation by FT-IR, FT-Raman, photoacoustic spectroscopy and X-ray diffraction techniques

The prepared complexes were characterised by FT-IR, FT-Raman and photoacoustic spectroscopy. Only the complex that was

prepared using the co-precipitation method was evaluated using the X-ray diffraction.

3.1.1. FT-IR

The FT-IR peak assignments of the curcumin spectrum are presented in Table 1. The curcumin, simple mixture of curcumin, β-CD, curcumin–β-CD complex (from co-precipitation) and β-CD spectra are shown in Fig. 1A.

In the curcumin spectrum, there were no bands in the most significant carbonyl region (1800–1650 cm^{-1}), indicating that curcumin exists in the keto-enol tautomeric form. The spectrum of the simple mixture exhibited peaks that corresponded to both of the components that were present. In the spectrum of the curcumin–β-CD complex from co-precipitation, good evidence of complex formation was obtained. The peak at 856 cm^{-1} shifted towards 846 cm^{-1}, while the same peak for the simple mixture did not change. The peak at 1153 cm^{-1} shifted to 1157 cm^{-1}, which is the frequency that corresponds to C–O vibrations, i.e., the vibrations of functional groups present in the β-CD cavity. In this region, a shoulder was exhibited at 1144 cm^{-1}, which may be an indication of complexation. This shift also occurred for the simple mixture but without the shoulder at 1144 cm^{-1}. The peak at 1281 cm^{-1} split into three overlapping peaks between 1265 and 1295 cm^{-1}, indicating that some interaction occurred between β-CD and the ring on the enolic side of the curcumin molecule. This peak remained the same in the simple mixture spectrum. The β-CD molecule exhibited no peaks in this region.

The spectra region with the most significant variations is shown in Fig. 1B. The peak at 1510 cm^{-1}, which is due to the C=O stretching and CCC and CC=O bending, undergoes a shift to 1514 cm^{-1} for the curcumin–β-CD complex from co-precipitation, which is good evidence of complex formation. The same phenomenon occurs for the peak at 1602 cm^{-1}, which corresponds to the C=C stretching of the aromatic rings, and could be observed in the simple mixture spectrum. In the spectrum of the curcumin–β-CD complex from co-precipitation, the peak at 1602 cm^{-1} showed a shoulder at 1587 cm^{-1}. Therefore, the FT-IR technique enabled good evidence to be obtained for complex formation between β-CD and curcumin using the co-precipitation method. The interactions appeared to occur due to the entry of one or both of the aromatic rings of curcumin into the CD cavity. The results of this study corroborate with those obtained by Tang et al. (2002), who also complexed curcumin with β-CD using the co-precipitation method and found relevant shifts in the complex spectrum, i.e., at 1602 and 1281 cm^{-1}, which are assigned to aromatic ring vibrations.

The complexes that were prepared by freeze-drying and solvent evaporation did not exhibit significant spectral differences due to the low colourant content and to the high absorption of the β-CD molecule (data not shown). Mohan et al. (2012) concluded that FT-IR failed to explain the inclusion complex formation of curcumin with CD derivatives. Their complexes were prepared using the freeze-drying method and the curcumin content in the complexes was very low and the major peaks of curcumin were overlapped by the CD peaks. Yallapu et al. (2010) analysed the curcumin–β-CD complex that was prepared using the solvent evaporation method by FT-IR and observed that, in the inclusion complex spectrum, all of the peaks belonging to CD appeared and only a few of the curcumin peaks were visible. Due to complexation, all of the CD related peaks were shifted to higher or lower frequencies, thus confirming the presence of curcumin in the complex. However, their results were not enough to confirm the occurrence of complexation.

3.1.2. FT-Raman

With FT-IR, many bands of the β-CD spectrum can mask important bands of complex formation. However, according to Mohan et al. (2012), the Raman spectra of CDs have regions that can be used to monitor variations in curcumin vibrations because these regions are free of bands. Some examples are the regions of the stretching vibration of double bonds and of aromatic C–H bonds. Thus, this technique facilitates the evaluation of inclusion complex formation in collaboration with FT-IR analysis. The Raman peak assignments of the curcumin characteristic spectra are presented in Table 1.

The Raman spectra of curcumin, the simple mixture, the curcumin–β-CD complex from co-precipitation and β-CD are shown in Fig. 1C. The curcumin spectrum, as stated by other authors and as observed in the FT-IR results, suggests that curcumin exists in the keto-enol tautomeric form (Kolev, Velcheva, Stamboliyska, & Spiteller, 2005; López-Tobar et al., 2012).

The simple mixture spectrum was expected to be the sum of the curcumin and β-CD spectra. However, because the Raman intensities of β-CD are weak, the spectrum of the simple mixture appeared to be the same as the curcumin, which was also observed by Mohan et al. (2012). In the spectra of the curcumin–β-CD complex from co-precipitation, important changes were observed in several regions, indicating molecular interactions between curcumin and β-CD in the complex and corroborating the FT-IR results. The colourant peak at 1627 cm^{-1} shifted towards 1637 cm^{-1} in the spectrum of the curcumin–β-CD complex from co-precipitation, while, in the spectrum of the simple mixture, this peak remained at 1627 cm^{-1}. The intensity of the 1600 cm^{-1} peak (which is attributed to aromatic C=C stretching) was significantly reduced. The intensities of the peaks of curcumin, simple mixture and curcumin–β-CD complex from co-precipitation at 1600 cm^{-1} (I_{1600}) and 1627 or 1637 cm^{-1} (I_{1627} or I_{1637}) were compared. When the ratio I_{1627}/I_{1600} was calculated for curcumin and the simple mixture, the value was found to be 0.58 and 0.60, respectively. However, for the curcumin–β-CD complex ratio I_{1637}/I_{1600}, the value was 1.16. The cavity most likely restricted the aromatic

Table 1
The infrared and Raman peak assignments of curcumin according to Kolev et al. (2005) and Mohan et al. (2012).

Curcumin (cm^{-1}) IR	Curcumin (cm^{-1}) Raman	Peak assignment
856		γ(CH) of aromatic and skeletal CCH
1153	1151	δ(CCH) of aromatic rings and δ(C–OH) of the enolic group coupled to δ(C=CH) in the inter-ring chain
	1250	δ(CH) of the aromatic rings, combined to ν(C–O) of the ether groups linked to these rings
1281		δ(CH) of C=CH, ν(CCH) of the aromatic ring in the enolic side of the molecule
	1317	δ(CCH) of the inter-ring chain (C^{10} and C^{11})
	1430	δ(CCC), δ(CCH) and δ(C–OH) of aromatic rings
1510		ν(C=O), δ(CCC) and δ(CC=O)
1602	1600	ν(C=C) of aromatic rings
1627	1627	ν(C=C) and ν(C=O) of the inter-ring chain

Vibrational modes: δ = in plane bending; γ = out of plane bending; ν = stretching.

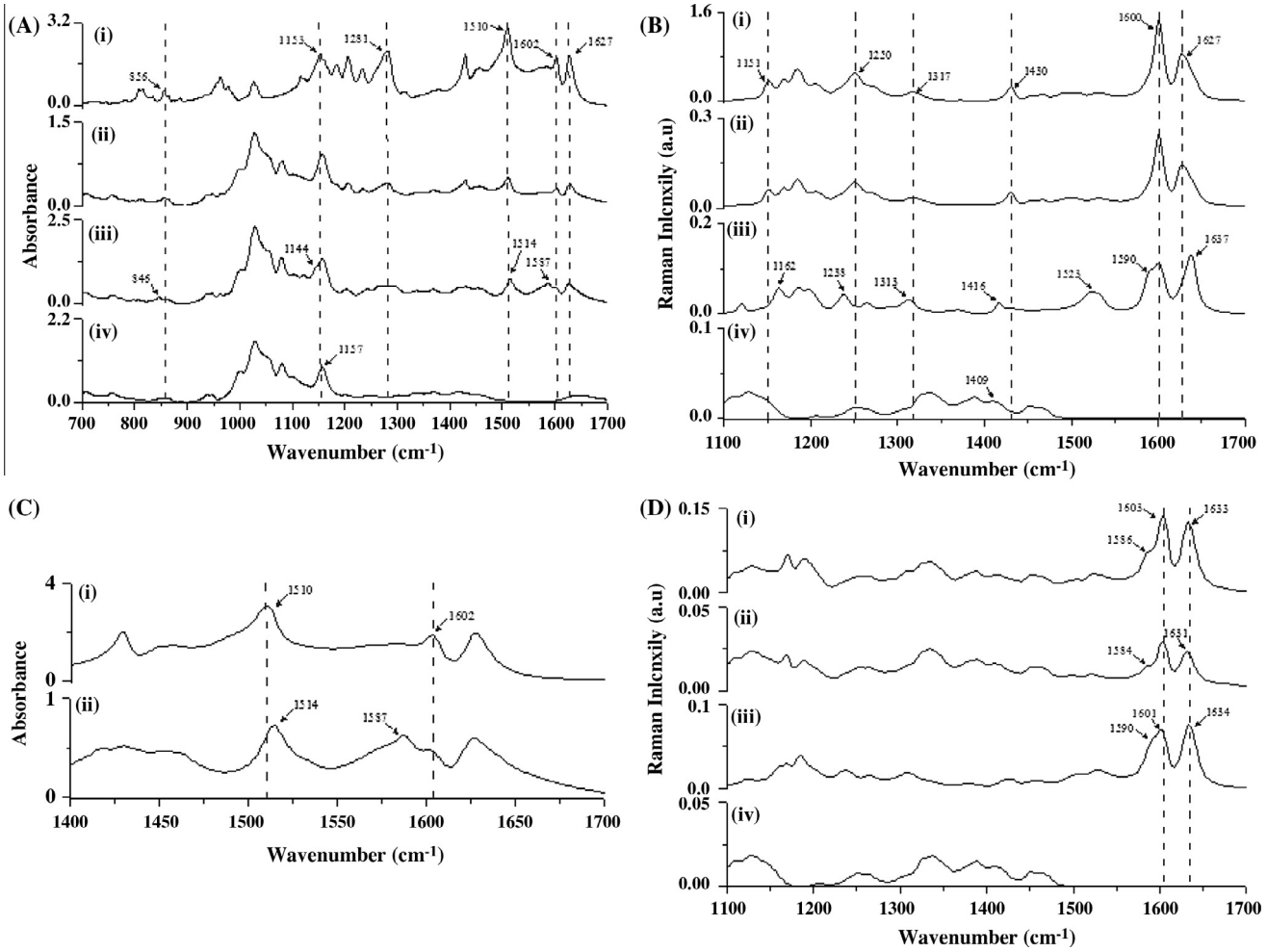

Fig. 1. FT-IR spectra of (i) curcumin, (ii) simple mixture of curcumin with β-cyclodextrin in a 1:2 molar ratio (iii) curcumin–β-cyclodextrin complex from co-precipitation and (iv) β-cyclodextrin (A); zoom in the 1400–1700 cm^{-1} region of Fig. 1A of (i) curcumin and (iii) curcumin–β-cyclodextrin complex from co-precipitation spectra (B); FT-Raman spectra of (i) curcumin, (ii) simple mixture of curcumin with β-cyclodextrin in a 1:2 molar ratio of (iii) curcumin–β-cyclodextrin complex from co-precipitation and (iv) β-cyclodextrin (C); FT-Raman spectra of (i) the curcumin–β-cyclodextrin complex from freeze-drying with curcumin diluted in ethanol, (ii) the curcumin–β-cyclodextrin complex from freeze-drying without the use of ethanol, (iii) the curcumin–β-cyclodextrin complex from solvent evaporation and (iv) β-cyclodextrin (D). The dashed lines show the curcumin peaks that suffered shifts or modifications.

vibrations in the spectra, leading to a decreased peak intensity. Also, a small shoulder was present at 1590 cm^{-1} in the curcumin–β-CD complex from co-precipitation spectrum, suggesting that one or both of the aromatic rings was inside the CD cavity.

Furthermore, other differences were observed in the spectrum of the curcumin–β-CD complex from co-precipitation when compared with the curcumin spectrum. The bands at 1430, 1250 and 1151 cm^{-1} were considerably shifted to 1416, 1258 and 1162 cm^{-1}, respectively, indicating interactions between β-CD and curcumin in the CCC, C–OH, C–H groups of the aromatic rings, in the ether groups that are linked to these rings and in the enolic group in the inter-ring chain. The band at 1317 cm^{-1} had a slight shift, but this shift was within the resolution (4 cm^{-1}), showing that there were no interactions in the central region of the molecule, i.e., in the C–C–H groups of the inter-ring chain, specifically C^{10} and C^{11}. The Raman and FT-IR results indicated that one or both ends of the curcumin molecule goes into the CD cavity and that the enolic and/or carbonylic part of curcumin undergoes H-bonding with the hydroxyl groups of CD. These results are very close to those described by Mohan et al. (2012), which performed curcumin complexation with CD derivatives and obtained considerable shifts in the same bands as in this work.

López-Tobar et al. (2012) were the first to use Raman spectroscopy for the study of the encapsulation of curcumin with β-CD. However, the curcumin spectrum was not affected by the presence of β-CD, suggesting that the colourant was poorly encapsulated by β-CD. These authors stated that a possible reason for the lack of complex formation was the large size of the curcumin extremity, i.e., the approximate width of the terminal aromatic part of curcumin is 7.2 Å, while the inner cavity of β-CD is in the range of 6.0–6.5 Å. However, Tang et al. (2002) stated that curcumin has a 19 Å length and 6 Å width, which enables the possibility of curcumin rings entering the β-CD cavity. The authors also affirmed that it is reasonable to consider complex formation with two molecules of β-CD because, according to the molecules dimensions, curcumin appears to be too large to be entirely included in one β-CD cage.

The curcumin–β-CD complexes from freeze-drying and from solvent evaporation also exhibited some changes in the Raman spectrum, similar to the curcumin–β-CD complex from co-precipitation but without the same shift intensities (Fig. 1D). Moreover, both of the curcumin–β-CD complexes from freeze-drying exhibited two wide regions of the spectrum in which no analysis could be conducted because the peaks were exactly the same as those found in the β-CD spectrum (1100–1160 and 1225–1500 cm^{-1}).

3.1.3. Photoacoustic spectroscopy

The absorption spectra of curcumin and the simple mixture obtained by photoacoustic spectroscopy showed different intensities

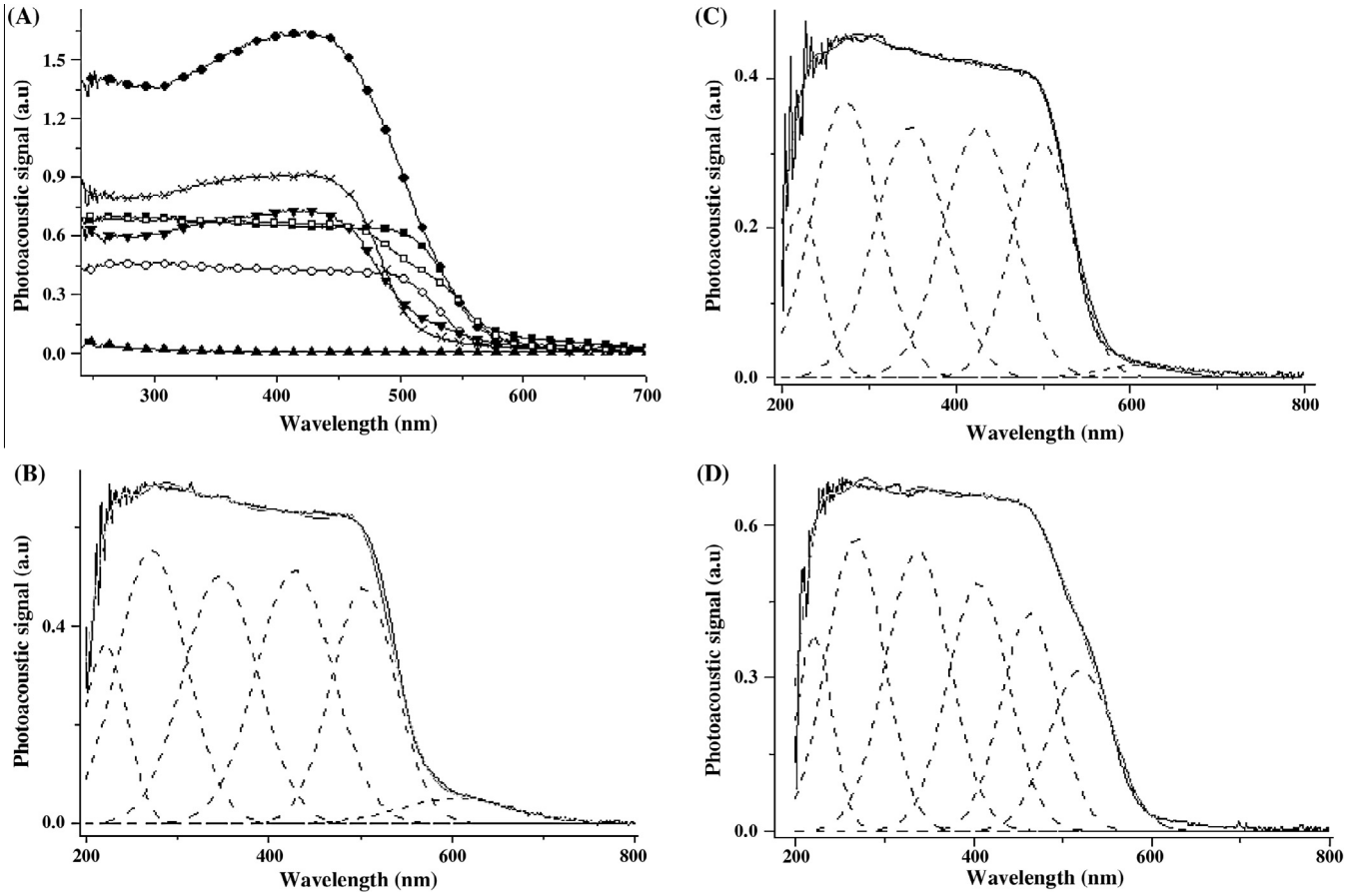

Fig. 2. Optical absorption spectra of (■) curcumin, (○) the simple mixture of curcumin with β-cyclodextrin in a 1:2 molar ratio, (▲) β-cyclodextrin, (●) the curcumin–β-cyclodextrin complex from solvent evaporation, (×) the curcumin–β-cyclodextrin complex from freeze-drying with curcumin diluted in ethanol, (▼) the curcumin–β-cyclodextrin complex from freeze-drying without the use of ethanol and (□) the curcumin–β-cyclodextrin complex from co-precipitation (A); Gaussian deconvolution of: curcumin optical absorption spectra (B); the simple mixture of curcumin with β-cyclodextrin in a 1:2 molar ratio optical absorption spectra (C); the curcumin–β-cyclodextrin complex from co-precipitation optical absorption spectra (D).

(Fig. 2A) due to the colourant "dilution" by β-CD. β-CD exhibited no signals in all of the analysed UV–Visible spectral region, which could facilitate the characterisation of the inclusion complexes. The spectrum of the curcumin–β-CD complex from co-precipitation was quite similar to the spectrum of curcumin up to 460 nm, at higher wavelength the complex absorption started to decrease. The spectra of the curcumin–β-CD complexes from freeze-drying were similar to the spectrum of the complex from solvent evaporation with a lower absorption up to 300 nm and following a signal increase. None of the samples exhibited signals at 800 nm. The spectra of the curcumin–β-CD complexes were not similar to the spectrum of the simple mixture; however, the spectra themselves were different, which complicates the understanding of the possible variations that are related to the molecular inclusion phenomena. The mathematical treatment used to identify variations in the spectra, that could be related to molecular inclusion, was Gaussian deconvolution. This procedure is based on using a sum of Gaussians functions to describe the experimental data. Fig. 2B–D shows the bands obtained by Gaussian deconvolution of the optical absorption spectra of curcumin, the simple mixture of curcumin and β-CD and curcumin–β-CD complex from co-precipitation, respectively.

The separation of the curcumin spectrum exhibited six Gaussian peaks, centered at 221, 272, 347, 428, 503 and 605 nm. The band at 428 nm is characteristic of curcumin extremities, it was assigned to the aromatic rings with their hydroxyl and ether groups (Crivello & Bulut, 2005). The separation of the simple mixture spectrum resulted in the same six peaks. However, the separation of the spectrum of the curcumin–β-CD complex from co-precipitation showed some shifting of peak centres. In particular, the peak at 428 nm did not appear, indicating the molecular inclusion of the curcumin rings in the β-CD cavity. The separation of the bands of the spectra of curcumin–β-CD complexes from freeze-drying and solvent evaporation exhibited the same profile of curcumin–β-CD complex from the co-precipitation spectrum.

3.1.4. X-ray diffraction

The curcumin–β-CD complex from co-precipitation was evaluated using X-ray diffraction (Fig. 3). The diffractograms of curcumin and β-CD exhibited a series of thin and intense lines, which are indicative of crystallinity.

The diffractogram of the simple mixture was the sum of the spectral lines of both of the components that were present, as expected. However, the diffractogram of the curcumin–β-CD complex from co-precipitation exhibited the disappearance of some of the curcumin spectral lines at 7.90°, 14.5°, 15.2°, 15.8° and 18.2° (2θ). Additionally, the appearance of new lines was observed, including weak lines at 5.83°, 6.58° and 6.91° (2θ) and an intense line at 14.1° (2θ), indicating the presence of new solid crystalline phases that correspond to an inclusion complex of the same nature. Thus, the X-ray diffraction corroborated the results that were obtained from FT-IR, FT-Raman and photoacoustic spectroscopy techniques for the curcumin–β-CD complex that was prepared by co-precipitation.

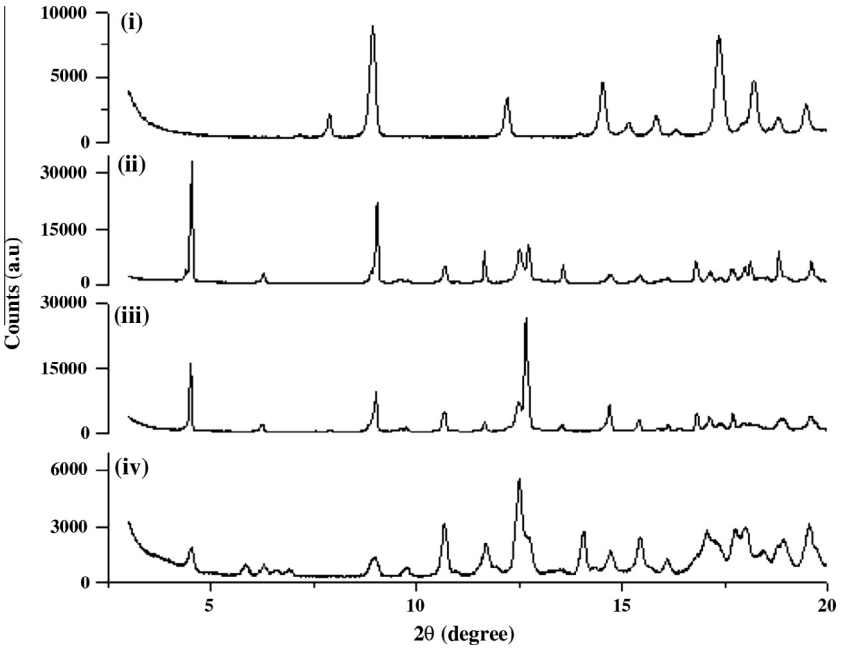

Fig. 3. X-ray diffraction patterns of (i) curcumin, (ii) β-cyclodextrin, (iii) the simple mixture of curcumin with β-cyclodextrin in a 1:2 molar ratio and (iv) the curcumin–β-cyclodextrin complex from co-precipitation.

3.2. Complexation efficiency and solubility of the curcumin–β-CD complexes

The complexation efficiency of the different methodologies that were used for complex preparation was determined. The curcumin–β-CD complex from co-precipitation had an efficiency value that was considerably higher than those of the other complexes, showing that 74% of the colourant quantity that was initially added to the process remained in the obtained complex. The second best methodology, solvent evaporation, had a complexation efficiency of 14% and the other methods had complexation efficiency values of less than 3%.

Paramera et al. (2011b) worked with the microencapsulation of curcumin using three encapsulants: yeast cells, β-CD and modified starch. In the β-CD complexes, the authors used different complexation methodologies, such as freeze-drying, co-precipitation, co-evaporation and kneading, and obtained low efficiency values, between 5.7% and 22.8%. The best result was for the freeze-drying methodology, followed by co-precipitation (17.1%). For their other encapsulants, the authors obtained good efficiency results, up to 60.4% for modified starch and up to 88.2% for yeast cells.

Wang et al. (2009) achieved great microencapsulation efficiency for curcumin microcapsules with gelatin using the spray-drying process, with values ranging between 73.2% and 98.4%.

According to Tonnesen et al. (2002), the solubility of curcumin in water is practically zero in acidic and neutral pH. Curcumin is soluble in alkali but, under these conditions, rapidly undergoes hydrolytic degradation, which limits its application. The complexation of curcumin with CDs can solve these problems.

The curcumin–β-CD complexes from co-precipitation and freeze-drying with curcumin diluted in ethanol exhibited the highest increase in colourant water solubility, compared with the pure colourant of 31- and 28-fold, respectively. The curcumin–β-CD complexes from solvent evaporation and freeze-drying without the use of ethanol exhibited an increase in colourant solubility of 19- and 18-fold, respectively. The curcumin–β-CD complex from co-precipitation had the highest amount of colourant due to its higher efficiency and was expected to have a much higher solubility than the other complexes. However, the solubility was low, indicating that part of the dye was in the free form; nevertheless, this solubility was the highest obtained, indicating the advantages of using this complex. The co-precipitation process is the simplest, fastest and is the cheapest of all of the used methodologies, enabling its use in food industry.

Other authors also obtained an improvement in curcumin solubility via the complexation of the molecule with CDs. Tonnesen et al. (2002) achieved an increase in curcumin solubility in the order of 10^4 for pH 5, after the addition of CD derivatives to the solution. Singh et al. (2010), who was also working with CD derivatives, obtained solubility values of 0.1 mg/ml using HP-β-CD and of 0.73 mg/ml using HP-γ-CD at pH = 6. Yallapu et al. (2010) reported that the pure curcumin solubility in phosphate buffered saline was approximately 20 μg/ml and an increase in solubility was achieved as the β-CD quantity was increased. Wang et al. (2009) obtained curcumin microcapsules in gelatin, using spray-drying, which were 100% water soluble (0.3% w/v).

Based on the obtained results from all of the complex characterisations, complexation efficiencies and solubility assays, the curcumin–β-CD complex from co-precipitation was selected for subsequent stability and food application tests. Marcolino et al. (2011) also obtained the best results for the co-precipitation technique, compared with kneading and simple mixing methods.

3.3. Stability studies

Curcumin, when exposed to UV/visible radiation, undergoes degradation, both in the liquid and solid states. The main degradation product is a cyclisation of curcumin, which is formed by the loss of two hydrogen atoms from the molecule. Vanillin, vanillic acid, ferulic aldehyde, ferulic acid and 4-vinylguaiacol are also formed (Tonnesen, Karlsen, & van Henegouwen, 1986). Curcumin complexation with CDs has been used by many authors to protect the molecule against photodegradation (Paramera et al., 2011b; Tonnesen et al., 2002; Wang et al., 2009).

After 30 days of sunlight exposure, the curcumin–β-CD complex exhibited colourant retention, i.e., a photostability 18% higher than

that of the pure colourant. The curcumin retention for the colourant was 72 ± 1% and for the complex was 84 ± 3% (Fig. 4A). This improvement in stability is due not only to the physical protection barrier but also to complexation effects because, when a simple mixture sample was submitted to the same treatment, it exhibited a curcumin retention of 73.4 ± 0.2% (data not shown).

Paramera et al. (2011b) studied the photochemical stability of curcumin encapsulated in yeast cells, in β-CD (using the freeze-drying method) and in modified starch. The authors noticed that, after a 30-day exposure to sunlight, the best result was for the yeast microcapsules, which had a colourant retention of 87.2 ± 0.3%. The retention of the non-encapsulated curcumin was 62.8 ± 0.2%. The authors also concluded that the β-CD complexes did not protect curcumin from photodegradation and enhanced its instability. In this work, unlike in Paramera et al. (2011b), the result obtained for the co-precipitation technique (Fig. 4A) was as positive as the one obtained for the yeast cell microcapsules.

According to Tonnesen et al. (2002), the inclusion complex formation causes an increase in guest photodegradation in some cases. The authors reported that curcumin complexation with CDs led to a photodegradation of the colourant in solution, most likely due to intermolecular hydrogen bond formation between curcumin and the CD molecules. This result corroborates the one obtained by Paramera et al. (2011b). However, this result was the opposite of that obtained in this work.

Wang et al. (2009), who was working on curcumin microencapsulation in gelatin via spray-drying, conducted light stability tests of their microcapsules in solution. The authors showed that after a 30-day light exposure, the absorbance of the solution with the microcapsules decreased by less than 1.5%, while the absorbance of the solution with the pure colourant decreased by almost 18%.

Fig. 4B shows the stability results of the pure and complexed curcumin between pH 1–9. The curcumin–β-CD complex exhibited better stability than the pure colourant for the pH range of 1–7, by approximately 2.7-fold. However, at pH values 8 and 9, degradation occurred for both the complex and the pure colourant. In addition, both solutions visually changed in colour from yellow to red. According to Tang et al. (2002), Tomren, Másson, Loftsson, and Tonnesen (2007) and Tonnesen et al. (2002), curcumin is unstable in an alkali medium because it is exposed to hydrolytic degradations. Under these conditions, the yellow colour of curcumin turns red (Wang et al., 2009). Tang et al. (2002) also observed that, in a strong alkali medium, the presence of β-CD has little effect on the absorbance of curcumin. However, in an acid medium, the colourant absorbance is increased. Wang et al. (2009) stated that the microencapsulation of curcumin in gelatin by spray-drying had better acid stability than the colourant in its pure form.

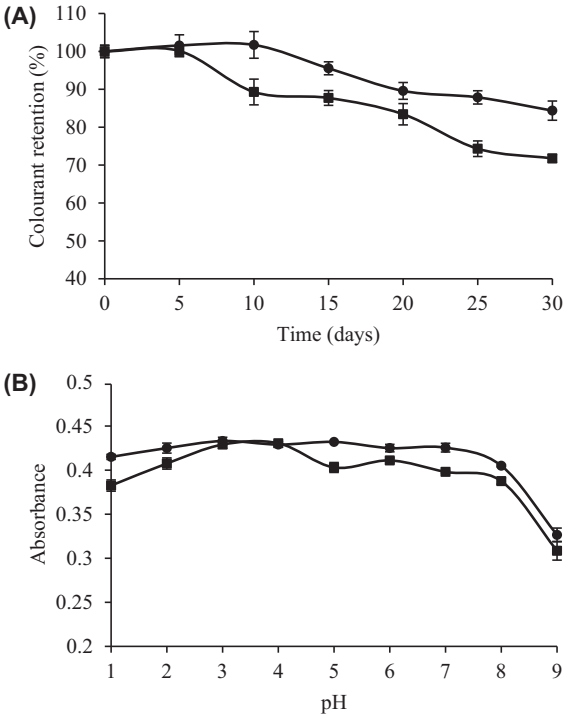

Fig. 4. Curcumin retention after 30 days of exposure to natural light (A); stability in different pHs (B). Pure curcumin (■) and the curcumin–β-cyclodextrin complex (●).

The storage stability of the pure colourant and of the curcumin–β-CD complex was assessed for 90 days at three different temperatures, −15, 4 and 25 °C (Fig. 5A1, B1 and C1). The colour retention values after 90 days at −15 °C were 87 ± 2% for the pure colourant and 95 ± 2% for the complex and at 4 °C were 86 ± 1% for the pure colourant and 89 ± 2% for the complex. Thus, the retention values with the use of β-CD were 9% better at −15 °C (which is a suitable temperature for the storage of the dye according to the manufacturer) and 4% better at 4 °C. The observed improvements are attributed to colourant complexation because the same assay was conducted for the simple mixture, and the retention results at −15 and 4 °C were 85 ± 2% and 82 ± 1%, respectively (data not shown). No improvement or variation between the pure and complexed colourant was obtained for the storage of the samples at 25 °C (Fig. 5C1).

Marcolino et al. (2011) performed simultaneous storage and light stability tests on pure curcumin and curcumin complexed with β-CD using co-precipitation, by storing the samples in the dark and under natural light. However, the complex formation between curcumin and β-CD did not improve the colourant stability against light and storage. The colour loss of the complex after 53 days of storage was very similar to the colour loss of the pure colourant (an approximate 40% decay in colour intensity) under both light conditions.

Paramera et al. (2011b) achieved storage stability results that were similar to those of the present study. However, the authors assessed the relative humidity effect and kept the storage temperature at 25 °C. According to the authors, the encapsulation of curcumin in β-CD enhanced the curcumin storage stability, especially at a lower relative humidity, but to a significantly low degree.

The thermal stability of pure curcumin and of the curcumin–β-CD complex was assessed after a 2 h isothermal heating at 100, 150 and 200 °C. At 100 and 150 °C, the pure colourant degradation was very low with a colour retention of 97.4 ± 0.8% at 100 °C and of 96 ± 1% at 150 °C. The curcumin–β-CD complex exhibited a subtle improvement in its stability. The obtained retention values were 99.3 ± 0.9% and 99.0 ± 0.6% at 100 and 150 °C, respectively (Fig. 5A2 and B2). At 200 °C (Fig. 5C2), the colour loss of the pure colourant was severe, and the retention reached the value of 28 ± 2%. This result is because the melting point of curcumin is between 170 and 180 °C; the melting point of curcumin is 172 °C according to Yallapu et al. (2010) and 176 °C according to Marcolino et al. (2011). Under the same conditions, the curcumin–β-CD complex exhibited a colourant retention of 32.9 ± 0.3%, showing it to be unstable at this temperature, even with its content 20% higher than that of the pure colourant.

Paramera et al. (2011b) obtained results that were similar to those of this study. At 100 °C and after 120 min of heating, curcumin was stable, and all of the encapsulation forms exhibited a similar stability. After 2 h of isothermal heating at 150 °C, there was a slight decrease in the stability of the colourant with β-CD, with a colour retention of 99.0 ± 0.6%. At 200 °C, their microcapsules had colour losses between 26.7 and 40.0%, while pure curcumin lost 42.1% of its colour.

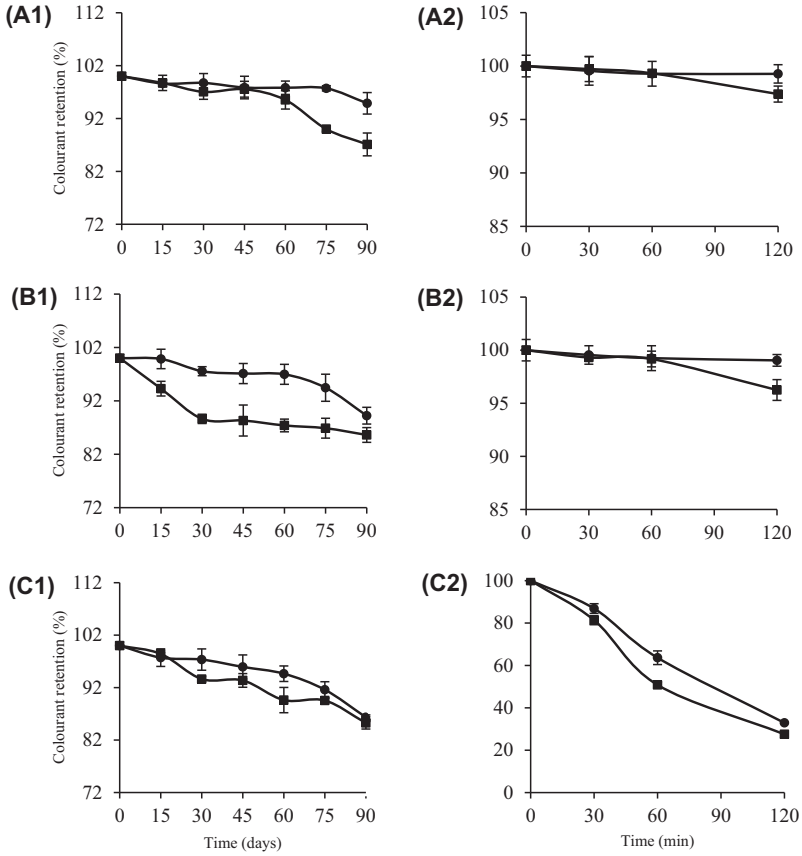

Fig. 5. Curcumin retention after 90 days of storage at −15 °C (A1), 4 °C (B1) and 25 °C (C1); curcumin retention after 2 h of an isothermal heating at 100 °C (A2), 150 °C (B2) and 200 °C (C2). Pure curcumin (■) and the curcumin–β-cyclodextrin complex (●).

Wang et al. (2009) assessed the heat stability of pure curcumin and of the microcapsules before and after the spray-drying process in solution. The authors found that the colour rapidly initiates its degradation at 70 °C. With an isothermal heating of the pure and microencapsulated colourant at 100 °C for 50 min, the absorbance of pure curcumin decreased by 25.9%, while the absorbance of the microcapsules before and after spray-drying decreased 5.9% and 2.8%, respectively.

3.4. Food application

Pure curcumin and the curcumin–β-CD complex from co-precipitation were used in the preparation of vanilla ice creams. The complex exhibited facilities in the preparation of the product comparing with the pure colourant, due to its better dispersion in the mixture. As described in Subsection 2.7, for the formulation that contained the pure colourant, vigorous stirring with a mixer was indispensable to accomplish the complete solubilisation of the colourant.

For the colorimetric analysis, formulations A, B and C had the same values for the L^* parameter. The results of the a^* axis revealed that the formulations had statistically different ($p \leqslant 0.05$) chromaticity in the axis, which goes from green (−) to red (+). The greenest formulation was B, followed by C and A. The results of the b^* axis and chroma showed that formulations A and C were equally yellow in colour and intensity and that formulation B had the most intense yellow colour (30.5 ± 0.7, 51 ± 6 and 31 ± 2 for the b^* axis, for formulations A, B and C, respectively; 31.3 ± 0.7, 52 ± 6 and 32 ± 2 for chroma, for formulations A, B and C, respectively). The colourant quantity that was used in formulation B was 83% higher than formulation C and was the same as that in formulation A. This result shows that curcumin complexation with β-CD intensifies the colour of the product. The b^* parameter and chroma increased by 67% and 66%, respectively, when formulations A and B were compared. Therefore, there is a significant economy in the preparation of the product with the complexed colourant because, although the quantity of the complex that was used to produce the same colour is 20% higher than the quantity of the pure colourant (formulations A and C), the pure curcumin cost is at least 150% higher than the β-CD cost, and formulation C contained 83% less curcumin than formulation A.

The hue angle was significantly different for all of the formulations (102.3 ± 0.7, 101 ± 1 and 105.6 ± 0.2 for formulations A, B and C, respectively), but these values were quite close. The three samples were located in the second quadrant (between 90° and 180°) with their values closer to yellow (90°) than to green (180°). Marcolino et al. (2011) used curcumin and the curcumin–β-CD complex from co-precipitation in minas fresh cheese and yogurt. The authors used 5 ppm of pure colourant and an equal quantity of the complex for the yogurt and used 20 ppm for the cheese. The obtained hue angle values were close for both products but were significantly different. For chroma, the values were very different for both products, showing a significant intensification in the colour of the product with the curcumin–β-CD complex.

The results obtained from the sensory evaluation of the ice creams showed that the use of the pure or complexed colourant caused no statistically differences ($p \leqslant 0.05$) in the texture, taste and flavour attributes of formulations A, B and C. In the opinions of the panellists, all of these attributes were well accepted and received average scores between 7.04 and 7.71 on a scale of 9 points. For the colour attribute, formulations A and C were equally accepted by the panellists, and the colourimeter (chroma parameter) showed that they had the same yellow colour intensity. These formulations showed better acceptance than formulation B, which

was considered by some panellists to be too dark to be characteristic of vanilla ice cream. The average scores for the colour attribute of the vanilla ice creams were 7.70, 6.03 and 7.45 for formulations A, B and C, respectively.

Colour is the first criterion that is used in the acceptance or rejection of a product. Thus, in the food industry, colour is an important attribute. If the colour is attractive, the product will be eaten or, at least, tasted. These affirmatives corroborate the buying intention results obtained. Formulations A and C had superior acceptances than formulation B, indicating that colour really is an attribute that influences the purchase of a food. The average scores for buying intention were 2.53, 2.12 and 2.56 for formulations A, B and C, respectively. Additionally, 58% of the panellists answered that they certainly would buy formulation A, while 66% would certainly buy formulation C, although both formulations had the same colourimetric analysis colour and the same sensorial acceptance.

4. Conclusion

The FT-IR, FT-Raman, photoacoustic spectroscopy and XRD results produced important evidence of curcumin–β-CD inclusion complex formation. In addition with the complexation efficiency and solubility results, these results indicated that co-precipitation was the best methodology used in this work for complexation. The colour stability of the curcumin–β-CD complex against sunlight, pH, storage and isothermal heating was higher than that of the pure colourant. The use of the curcumin–β-CD complex in vanilla ice creams intensified the colour of the products, because there were obtained higher values for the colourimetric parameters b^* and chroma, that are referred to as yellow colour and saturation. Also, the use of the complex in the ice creams had good sensorial acceptance by the panellists and promoted better dispersion in the prepared product compared with the pure colourant when a colourant quantity of 83% less than the pure colourant quantity was used. The use of the complex results in potential savings and in a consequent viability of its use in food industry.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2013.12.067.

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