UNIVERSIDADE ESTADUAL DE MARINGÁ

CENTRO DE CIÊNCIAS AGRÁRIAS

Programa de Pós-Graduação em Ciência de Alimentos

COMPLEXO DE INCLUSÃO CURCUMINA-β-CICLODEXTRINA: ESTABILIDADE, SOLUBILIDADE E CARACTERIZAÇÃO POR FT-IR, FT-RAMAN, DIFRAÇÃO DE RAIOS-X E ESPECTROSCOPIA FOTOACÚSTICA

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Dissertação apresentada ao programa de Pós-Graduação em Ciência de Alimentos da Universidade Estadual de Maringá, como parte dos requisitos para obtenção do título de mestre em Ciência de Alimentos.

Maringá

2013

Orientadora

Prof.ª Dr.ª Graciette Matioli

BIOGRAFIA

Camila Sampaio Mangolim nasceu em 08 de fevereiro de 1988 na cidade de Paranavaí, Paraná. Possui graduação em Engenharia de Alimentos pela Universidade Estadual de Maringá. Tem experiência na área de Biotecnologia, atuando principalmente nos seguintes temas: produção de ciclodextrinas e suas aplicações em alimentos.

Dedico

Aos meus pais, Olidio e Joselita, que são meu alicerce, meu exemplo, minha vida, os grandes responsáveis por eu ter me tornado a pessoa que sou hoje. À minha irmã, Amanda, meu orgulho, a melhor amiga que Deus poderia ter me dado, que se Ele me permitisse escolher, com certeza eu não o teria feito tão bem.

AGRADECIMENTOS

A Deus, Senhor de todas as coisas, que sempre me mostrou o caminho, principalmente nos momentos de maiores incertezas e inseguranças.

À minha família, pessoas que eu mais amo neste mundo. Meu pai Olidio, um exímio conselheiro, minha mãe Joselita, que sempre me incentivou a correr atrás dos meus objetivos, minha irmã Amanda, meu grande exemplo de responsabilidade. Obrigada também por me acompanharem nos experimentos fora de hora.

À minha orientadora, Prof.ª Graciette, pela competência, paciência, confiança e amizade durante todos esses anos de trabalho.

À minha avó Neli, uma senhora pequena em tamanho, mas grande no amor e carinho dedicado aos netos.

Aos meus tios e primos, pelo carinho, pelas alegrias, por estarem sempre aqui nos momentos importantes.

Aos meus amigos, pela atenção, carinho, conversas, auxílios e tudo o que compartilhamos.

A todos os meus colegas de laboratório, obrigada pelos trabalhos, auxílios e risadas compartilhadas durante este período.

Aos meus ex-colegas de trabalho na Lowçucar, que sempre me valorizaram como profissional, especialmente à Eliana, que se tornou uma grande amiga.

Aos professores e alunos de outros departamentos, em especial aos professores Mauro, Francielle e Medina do Departamento de Física, e Grasiele e Antônio do Departamento de Engenharia de Alimentos, pelo tempo dispensado a esta pesquisa. À aluna de pós-graduação em Física, Ana Claudia, pela parceria e pela amizade e ao aluno da mesma pós-graduação Leandro, pelas ideias e auxílios. Aos alunos de Engenharia de Alimentos, pelo auxílio no preparo dos produtos e análise sensorial.

Aos servidores da UEM, que tornam possível o desempenho das nossas atividades, em especial à dona Nina, que é também uma grande amiga.

E como não poderia faltar, às minhas pequenas “meninas”, as cachorrinhas Jully, Lully e Pity, pelas alegrias em todos os momentos dessa caminhada.

APRESENTAÇÃO

Esta dissertação de mestrado está apresentada na forma de um artigo científico:

AUTORES: Camila Sampaio Mangolim, Cristiane Moriwaki, Ana Claudia Nogueira, Mauro Luciano Baesso, Antônio Medina Neto e Graciette Matioli.

TÍTULO: Curcumin-β-cyclodextrin inclusion complex: stability, solubility and characterisation by FT-IR, FT-Raman, X-ray diffraction and photoacoustic spectroscopy.

REVISTA: Food Chemistry.

RESUMO GERAL

INTRODUÇÃO. A indústria alimentícia utiliza usualmente corantes artificiais para manutenção da cor original no produto processado e armazenado, pois a cor é um atributo que influencia de forma decisiva a preferência do consumidor ao adquirir o alimento. A opinião pública é muito sensível ao emprego de aditivos artificiais, demonstrando preferência pelos corantes naturais, devido às suas características nutricionais e de saúde. Porém, os corantes naturais, na grande maioria, são sensíveis ao pH, aquecimento, luz solar, oxigênio e pouco solúveis em água. Uma das possibilidades de estabilização destes corantes é a formação de complexos de inclusão com as ciclodextrinas (CDs). As CDs são maltooligossacarídeos cíclicos contendo seis, sete ou oito unidades de glucose unidas por ligações glicosídicas α-(1,4), chamadas α-, β- e γ-CD, respectivamente. São obtidas a partir do amido pela ação da enzima ciclodextrina glicosil-transferase (CGTase).

OBJETIVOS. O objetivo deste trabalho foi complexar a curcumina, um corante natural utilizado em alimentos e preparações farmacêuticas, com a β-CD por diferentes métodos para melhorar sua estabilidade a fatores ambientais desfavoráveis e facilitar sua aplicação em alimentos. Várias técnicas foram utilizadas visando à caracterização dos possíveis complexos formados.

MATERIAL E MÉTODOS. Os complexos de inclusão entre a curcumina e a β-CD foram preparados por métodos de co-precipitação, liofilização e evaporação do solvente. Todos foram caracterizados pelas técnicas de espectroscopia de infravermelho por transformada de Fourier (FT-IR), espectroscopia de infravermelho de espalhamento Raman por transformada de Fourier (FT-Raman) e espectroscopia fotoacústica. A técnica de difração de raios-X (DRX) foi utilizada para caracterizar apenas o complexo preparado por co-precipitação. A eficiência da complexação e solubilidade de todos os complexos foram avaliadas. O complexo preparado por co-precipitação foi utilizado para os testes de estabilidade à luz, pH, armazenamento e aquecimento. O ensaio de aplicação em alimentos foi realizado em sorvetes de creme e testes sensoriais e colorimétricos foram empregados na avaliação dos produtos elaborados com o possível complexo e com o corante puro.

RESULTADOS E DISCUSSÃO. As técnicas de caracterização mostraram bons indícios de formação de complexo de inclusão curcumina-β-CD, principalmente para o complexo preparado por co-precipitação. Nas técnicas de FT-IR e FT-Raman, relevantes deslocamentos dos picos referentes aos anéis aromáticos da curcumina ocorreram, especialmente no lado enólico da molécula, mostrando que um ou ambos os seus anéis foram incluídos na cavidade da β-CD. Na técnica de espectroscopia fotoacústica, a deconvolução gaussiana permitiu observar o desaparecimento da banda referente ao anel aromático da curcumina com os grupos éter e hidroxila nos espectros de absorção ótica de todos os complexos preparados, evidenciando a inclusão dos anéis da curcumina na cavidade da β-CD. A técnica de DRX também mostrou resultados de confirmação favoráveis para o possível complexo preparado por co-precipitação. A metodologia de co-precipitação proporcionou um aumento de solubilidade de 31 vezes para o complexo curcumina-β-CD em relação ao corante puro e, também, uma eficiência da complexação de 74%, que foi superior às demais metodologias avaliadas. Portanto, para os demais ensaios de estabilidade e aplicação em alimentos, foi utilizado o complexo preparado por co-precipitação.

As análises de estabilidade mostraram que a inclusão da curcumina na β-CD protegeu a molécula quando exposta a todos os fatores ambientais desfavoráveis. Quando exposto à luz solar o complexo proporcionou uma melhora de 18% na estabilidade do corante. Melhoras também ocorreram com a exposição à variações de pH, bem como quando submetido ao armazenamento a -15 e 4 °C. Quando submetido ao aquecimento isotérmico, especialmente a 100 e 150 °C, o corante complexado mostrou retenção da cor de aproximadamente 99%.

A aplicação do complexo curcumina-β-CD nos sorvetes de creme provocou grande intensificação de cor nos produtos, pois foram obtidos valores superiores para os parâmetros colorimétricos b* e croma, que são referentes à coloração amarela e sua saturação. As formulações contendo corante puro e o complexo curcumina-β-CD obtiveram ótima aceitação sensorial, contudo na formulação contendo o complexo curcumina-β-CD a quantidade de curcumina utilizada foi 83% menor que a formulação com corante puro.

CONCLUSÕES. Os resultados de caracterização obtidos por FT-IR, FT-Raman e os de eficiência da complexação e solubilidade, indicaram que a co-precipitação foi o melhor método para complexação. O complexo preparado por co-precipitação obteve resultados de confirmação favoráveis também pelas técnicas de espectroscopia fotoacústica e DRX. Quando usado para análises de estabilidade, este complexo provou que a inclusão da curcumina na β-CD protegeu a molécula quando exposta à luz solar, variações de pH, armazenamento e aquecimento. Quando aplicado em sorvetes de creme o complexo apontou maior facilidade de preparação do produto em relação ao emprego do corante puro, devido a sua melhor dispersão na mistura. Sua aplicação resultou numa intensificação da cor amarela do produto. Uma ótima aceitação pelos provadores, tanto do sorvete com complexo quanto aquele com corante puro, foi constatada, contudo, considerando que a quantidade de curcumina utilizada na formulação contendo o complexo foi 83% menor que a formulação com corante puro, a utilização do complexo resulta numa possível economia e consequente viabilização da sua utilização na indústria de alimentos.

Palavras chaves: curcumina, ciclodextrina, corante natural, inclusão molecular, técnicas de confirmação de inclusão molecular, co-precipitação.

GENERAL ABSTRACT

INTRODUCTION. The food industry often uses artificial colourants to maintain the original colour of processed and stored products, because colour is an attribute that decisively influences the preference of the consumer to buy any food. Public opinion is very sensitive to the use of artificial additives, showing preferences for natural colourants, due to their health and nutritional characteristics. However, most natural dyes are sensitive to pH, heating, sunlight, oxygen and are low water soluble. One of the possibilities to stabilise these colourants is the inclusion complex formation with cyclodextrins (CDs). CDs are cyclic maltooligosaccharides having six, seven or eight glucose units linked by α-(1,4)-glucosidic bonds, named α-, β- and γ-CD, respectively. They are obtained from starch via the action of the enzyme cyclodextrin glycosyl transferase (CGTase).

AIMS. The aim of this work was to form inclusion complexes of curcumin, a natural colourant used in food and pharmaceutical preparations, with β-CD using different methodologies to enhance its stability against unfavorable environmental factors and to facilitate its food application. Various techniques were used aiming the characterisation of the possible complexes formed.

MATERIAL AND METHODS. The inclusion complexes between curcumin and β-CD were prepared using the co-precipitation, freeze-drying and solvent evaporation methods. All the complexes were characterised using the Fourier transform infrared spectroscopy (FT-IR), Fourier transform Raman scattering infrared spectroscopy (FT-Raman) and photoacoustic spectroscopy techniques. The X-ray diffraction (XRD) technique was used only to characterise the complex formed using the co-precipitation method. The complexation efficiency and solubility of all complexes were evaluated. The complex prepared by co-precipitation was used to assess stability against light, pH, storage and isothermal heating. The food application assay was made in vanilla ice creams and sensory and colourimetric tests were employed in the evaluation of the products using the possible complex formed and the pure colourant.

RESULTS AND DISCUSSION. The characterisation techniques exhibited good evidence of curcumin-β-CD complex formation, mainly to the complex prepared using co-precipitation methodology. In the FT-IR and FT-Raman techniques, relevant shifts occurred in the peaks assigned to the aromatic rings of curcumin, especially on the enolic side of the molecule, indicating that one or both rings of the molecule were included in the β-CD cavity. In the photoacoustic spectroscopy technique, the Gausssian deconvolution enabled the evidenciation of the disappearance of the band assigned to the aromatic rings of curcumin with their ether and hydroxyl groups in the optical absorption spectra of all complexes prepared, showing the inclusion of the curcumin rings in β-CD cavity. XRD technique also indicated favorable confirming results for the possible complex prepared using co-precipitation. The co-precipitation method also produced an increase in solubility of 31-fold for the curcumin-β-CD complex compared with the pure colourant, and a complexation efficiency of 74%, that was higher than the other methodologies evaluated. Therefore, in the assays of stability and food application, the complex prepared using co-precipitation was used.

The stability assays exhibited that the inclusion of curcumin in β-CD protected the molecule against all unfavorable environmental factors. When exposed to sunlight, the complex provided an improvement of 18% in the stability of the colourant. Some

improvements also occurred with the exposition to pH variations and after storage at -15 and 4 °C. When submitted to an isothermal heating, especially at 100 and 150 °C, the complexed colourant exhibited colour retention of approximately 99%.

The application of the curcumin-β-CD complex in vanilla ice creams produced an intensification of the colour in the products, because there were obtained higher values for the colourimetric parameters b* and chroma, that are referred to yellow colour and saturation. The formulations containing the pure colourant and the curcumin-β-CD complex obtained great sensory acceptance. However, in the formulation that contained the curcumin-β-CD complex, a colourant quantity of 83% less than the pure colourant quantity was used.

CONCLUSIONS. The characterisation results obtained using FT-IR and FT-Raman, and the results of complexation efficiency and solubility indicated that co-precipitation was the best method for complexation. The curcumin-β-CD complex using co-precipitation had favorable confirming results also using photoacoustic spectroscopy and XRD techniques. When it was used in stability assays, this complex showed that the inclusion of curcumin in β-CD protected the molecule against sunlight, pH variations, storage and isothermal heating. When applied in 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. Its application led to an intensification of the yellow colour of the product. A great sensory acceptance by the panelists, for both ice creams prepared with the pure or the complexed curcumin, was observed. However, considering that the quantity of curcumin used in the formulation containing the complex was 83% lower than the formulation containing the pure colourant, the use of the complex results in potential savings and in a consequent viability of its use in food industry.

Key words: curcumin, cyclodextrin, natural colourant, molecular inclusion, molecular inclusion confirmation techniques, co-precipitation.

ARTICLE

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

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Abstract

Curcumin, a natural colourant that is used in food and pharmaceutical preparations, was complexed with β-CD using the co-precipitation, freeze-drying and solvent evaporation methodologies. 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, by the photoacostic spectroscopy with Gaussian deconvolution via the disappearance of the band related to aromatic rings and by the XRD technique via the modifications in the spectral lines. 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 to storage at -15 and 4 °C. With an isothermal heating at

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100 and 150 °C, 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 when a colourant quantity of 83% less than the pure colourant quantity was used.

Highlights:

Curcumin-β-CD complex using co-precipitation enhanced its solubility 31-fold. ► Curcumin-β-CD complex exhibited higher sunlight, pH, storage and heating stability. ► The FT-IR, FT-Raman, photoacoustic spectroscopy and XRD techniques produced important evidence of curcumin-β-CD complexation. ► The use of the complex in vanilla ice creams results in good sensorial acceptance and potential savings.

Key words: Curcumin, cyclodextrin, natural colourant, molecular inclusion, spectroscopic techniques, co-precipitation.

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 (Wang, Lu, Lv & Bie, 2009; Anand, Kunnumakkara, Newman & Aggarwal, 2007). 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 because of its innumerous pharmacological applications including anti-inflammation, anti-human immunodeficiency virus, anti-microbial, anti-oxidant, anti-parasitic, anti-mutagenic and anti-cancer (Yallapu, Jaggi & Chauhan, 2010; Singh, Tonnesen, Vogensen, Loftsson & Másson, 2010; Mohan, Sreelakshmi, Muraleedharan & Joseph, 2012). It is considered safe for human use, even in high doses (Singh et al., 2010; Anand et al., 2007).

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, and microencapsulation is a technique that is commonly used to overcome these

disadvantages (Wang et al., 2009; Paramera et al., 2011a; Paramera et al., 2011b; López-Tobar, Blanch, Castillo & Sanchez-Cortes, 2012).

The encapsulation of curcumin has been described in the literature using different materials. Among others, gelatin (Wang et al., 2009), cyclodextrins (CDs) (Szente, Mikuni, Hashimoto & Szejtli, 1998; Tonnesen, Másson & Loftsson, 2002; Mohan et al., 2012), cationic micelles (Leung, Colangelo & Kee, 2008), liposomes (Li, Braiteh & Kurzrock, 2005), yeast cells (Paramera et al., 2011a), and modified starch (Yu & Huang, 2010; Paramera et al., 2011b) are some of the most used encapsulating agents. In this context, CDs offer advantages over the other materials because they possess a hydrophobic cavity in which a wide variety of lipophilic guest molecules can be hosted. They are cyclic oligosaccharides having six, seven or eight glucose units linked by α-(1,4)-glucosidic bonds, named, respectively, α-, β- and γ-CD. 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 (Yallapu et al., 2010; Mohan et al., 2012). Yallapu et al. (2010) and Mohan et al. (2012) 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 (Mohan et al., 2012; López-Tobar et al., 2012), this technique was not successfully used to characterise curcumin-β-CD inclusion complexes. The photoacoustic spectroscopy is an innovative and low-cost technique that can also be valuable for investigation of complex formation between β-CD and guest molecules (Dias, Berbicz, Pedrochi, Baesso & Matioli, 2010).

The aim of this work is 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 the co-precipitation, freeze-drying and solvent evaporation techniques.

2.2.1. Co-precipitation

The Marcolino et al. (2011) method 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 and was rota-evaporated to remove ethanol. 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 around 50-55 °C and stocked for further measurements.

2.2.2. Freeze-drying

The Mohan et al. (2012) method 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 Yallapu et al. (2010) method was used. β-CD was dissolved in 8 mL of water in a glass vial with a magnetic bar. While this solution was stirred, 16.9 mg of curcumin dissolved in 500 µL of acetone were 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 stocked 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, and 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 the 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 by 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 its total amount added initially:

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

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 mixture 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 to an excitation at 1064 nm with 5 up to 200 mV. All of the spectra were an average of 1000 scans with a 4 cm^{-1} resolution.

The photoacoustic measurements of curcumin, β-CD, simple mixture 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 respect to the carbon black signal. The spectra were analysed by Gaussian deconvolution.

The X-ray diffractograms of curcumin, β-CD, simple mixture 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 of the 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 in Paramera et al. (2011b) with a minor modification. For one month, 160 mg of pure curcumin and 1.14 g of the curcumin-β-CD complex with the 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 subsection 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 subsection 2.3.

2.6.4. Thermal stability

The thermal stability of pure and complexed curcumin was assessed in accordance with the Paramera et al. (2011b) method 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 subsection 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. Two formulations were prepared that contained 2.23 g and 0.30 g of the curcumin-β-CD complex with the molar ratio of 1:2, and one formulation was prepared that contained 0.25 g of the pure colourant.

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 a vanilla flavouring, and the pure or complexed colourant into 400 mL of warm ultra high temperature (UHT) whole milk. 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 the laboratory scale, in individual booths under white light and with 80 untrained panelists. 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).

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 cordinates (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):

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

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

2.8. Statistical analysis

Data were evaluated by variance analysis (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 the 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 by the X-ray diffraction technique.

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 and β-CD, curcumin-β-CD complex from co-precipitation and β-CD spectra are shown in Fig. 1A.

Table 1

Figure 1

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 correspond 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, and 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 for complex formation between β-CD and curcumin using the co-precipitation method to be obtained. 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 the ones obtained by Tang et al. (2002), who also complexed curcumin with β-CD using the co-precipitation method and found relevant shifts in 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 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, then 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 technique

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

In the FT-IR technique, 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, simple mixture, 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 et al., 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, 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 misture 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 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 vibrations in the spectra, leading to a decrease in 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 went inside the CD cavity.

Furthermore, other differences were observed in the spectrum of the curcumin-β-CD complex from co-precipitation 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 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 molecule dimension, curcumin appears 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 cm^{-1} and 1225-1500 cm^{-1}).

3.1.3. Photoacoustic spectroscopy

The absorption spectra of curcumin and of the simple mixture that were obtained from photoacoustic spectroscopy appeared to be equal but had different intensities (Fig. 2A) due to the colourant “dilution” by β-CD. β-CD exhibited no signals in all of the

analysed UV-visible region, which could facilitate the characterisation of the inclusion complexes. The spectrum of the curcumin-β-CD complex from co-precipitation was very similar to the spectrum of curcumin until 460 nm, at which 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 until 300 nm and with an increase that started at this frequency. All of the samples exhibited no 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 strategy used to identify variations in the spectra that could be related do molecular inclusion was to separate the optical absorption bands, using Gaussian deconvolution. Figure 2B, 2C and 2D shows the bands obtained by Gaussian deconvolution of the optical absorption spectra of curcumin, simple mixture of curcumin and β-CD and curcumin-β-CD complex from co-precipitation, respectively.

The separation of curcumin spectrum exhibited six bands, with their centers in 221, 272, 347, 428, 503 and 605 nm. The band at 428 nm is characteristic of curcumin extremities, because it is assigned to the aromatic rings with their hydroxyl and ether groups. The separation of simple mixture spectrum gave the same six bands. However, the separation of the spectrum of curcumin-β-CD complex from co-precipitation showed some centers of the six bands shifted. Especially the band at 428 nm did not appear, indicating again the molecular inclusion of the curcumin rings in 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 co-precipitation spectrum.

Figure 2

3.1.4. X-ray diffraction

The curcumin-β-CD complex from co-precipitation was evaluated using the X-ray diffraction technique (Fig. 3B). 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 7.899, 14.544, 15.184, 15.842 and 18.209^{o} (2θ). Additionally, the appearance of new lines was observed, including weak lines at 5.833, 6.583 and 6.912^{o} (2θ) and an intense line at 14.087^{o} (2θ), indicating the presence of new solid crystalline phases that correspond to an inclusion complex of the same nature. Thus, the X-ray diffraction technique corroborated the results that were obtained from FT-IR, FT-Raman and photoacoustic spectroscopy techniques for the curcumin-β-CD complex that was prepared from co-precipitation.

Figure 3

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 acid 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 increases in colourant water solubility compared with the pure colourant, 31- and 28-fold, respectively. The curcumin-β-CD complexes from solvent evaporation and freeze-drying without the use of ethanol exhibited increases 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 and fastest and has the lowest cost of all of the used methodologies, enabling its use in food industry.

Other authors also obtained good improvement in curcumin solubility via the complexation of the molecule with CDs. Tonnesen et al. (2002) achieved an increase in curcumin solubility on 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 for pH = 6. Yallapu et al. (2010) reported that the pure curcumin solubility in phosphate buffered saline was approximately 20 µg/mL and achieved an increase in solubility as the β-CD quantity was increased. Wang et al. (2009) obtained curcumin microcapsules in gelatin by spray-drying 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 product of curcumin that 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 & Henegouwen, 1986). Curcumin complexation with CDs has been

used by many authors to protect the molecule against photodegradation (Paramera et al., 2011b; Wang et al., 2009, Tonnesen et al., 2002).

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 physical barrier protection 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).

Figure 4

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 sunlight exposure, the best result was for the yeast microcapsules, which had a colourant retention of 87.2 ± 0.3%. The retention of 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 less than 1.5%, while the absorbance of the solution with the pure colourant decreased almost 18%.

Fig. 4B shows the stability results of pure and complexed curcumin for the pH range of 1-9. The curcumin-β-CD complex exhibited better stability than the pure colourant for the pH range of 1-7, approximately 2.7-fold. However, for the 8 and 9 pH values, degradation occurred for both the complex and the pure colourant and both solutions visually changed their colour from yellow to red. According to Tomren, Másson, Loftsson and Tonnesen (2007), Tonnesen et al. (2002) and Tang 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.

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 ºC 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).

Figure 5

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 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 at 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 also 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.

Wang et al. (2009) assessed the heat stability of pure curcumin and of 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 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. Visual tests and colourimetric measurements were performed to determine the quantity of pure colourant that was needed to obtain the commercial vanilla ice cream colour. The needed quantity of the pure colourant in 1 L of UHT whole 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 sample A, i.e., 300 ppm (formulation C).

Formulations A, B and C had the same values for the L* parameter. The results of the a* axis revealed that the formulations had different 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 (Table 2). The colourant quantity that was used in formulation B was 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 underwent increases of 67% and 66%, respectively. 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 150% higher than the β-CD cost, and formulation C contained 83% less curcumin than formulation A.

The hue angle had significantly different values for all of the formulations, but these values were quite close (Table 2). The three samples were located in the second quadrant (between 90 and 180^{o}) with their values closer to yellow (90^{o}) than to green (180^{o}). 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 differences in the texture, taste and flavour attributes of formulations A, B and C (Table 3). In the opinions of the panelists, all of these attributes were well accepted and received average scores between 6.03 and 7.71 on a scale of 9 points. For the colour attribute, formulations A and C were equally accepted by the panelists (Table 3) and the colourimeter (chroma parameter) showed that they had the same yellow colour intensity (Table 2). These formulations showed better acceptance than formulation B, which was considered by some panelists to be too dark to be characteristic of vanilla ice cream.

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 that are presented in Table 3. Formulations A and C had superior acceptances than formulation B, indicating that colour really is an attribute that influences the purchase of a food. Additionally, 58% of the panelists answered that they certainly would buy formulation A, while 66% would certainly buy formulation C (data not shown), although both formulations had the same colourimetric analysis colour and the same sensorial acceptance.

4. Conclusions

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, had good sensorial acceptance by the panelists and promoted better dispersion in the prepared product compared with the pure colourant.

Acknowledgements

The authors are thankful to the Brazilian Agencies CAPES, CNPq, and Fundação Araucária for their financial support of this work.

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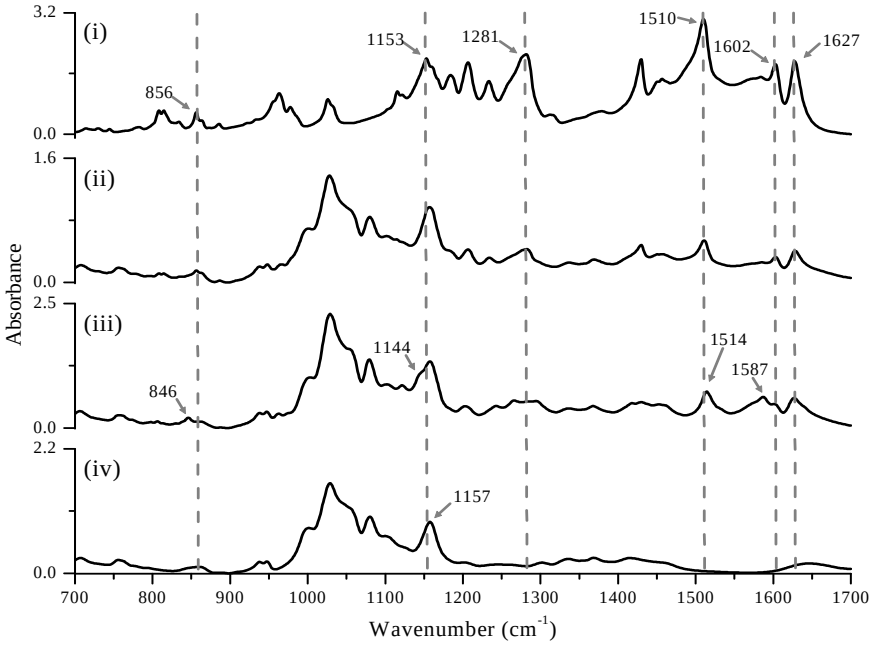
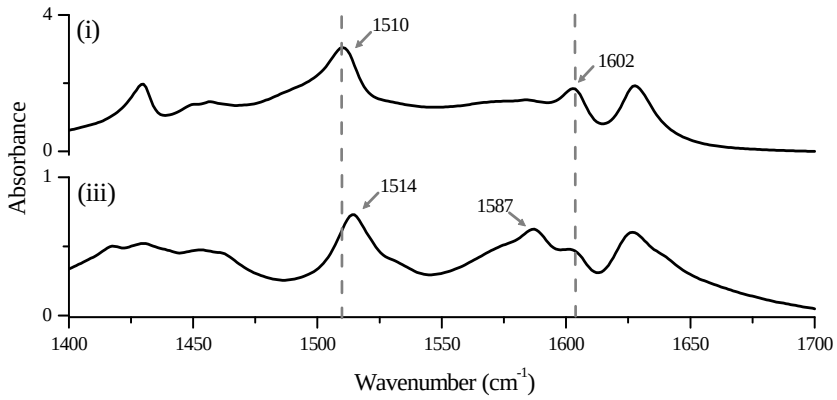
(A)


(i)
(ii)
(iii)
(iv)
856
1153
1281
1510
1602
1627
846
1144
1514
1587
1157
Absorbance
Wavenumber (cm-1)

(B)


(i)
(iii)
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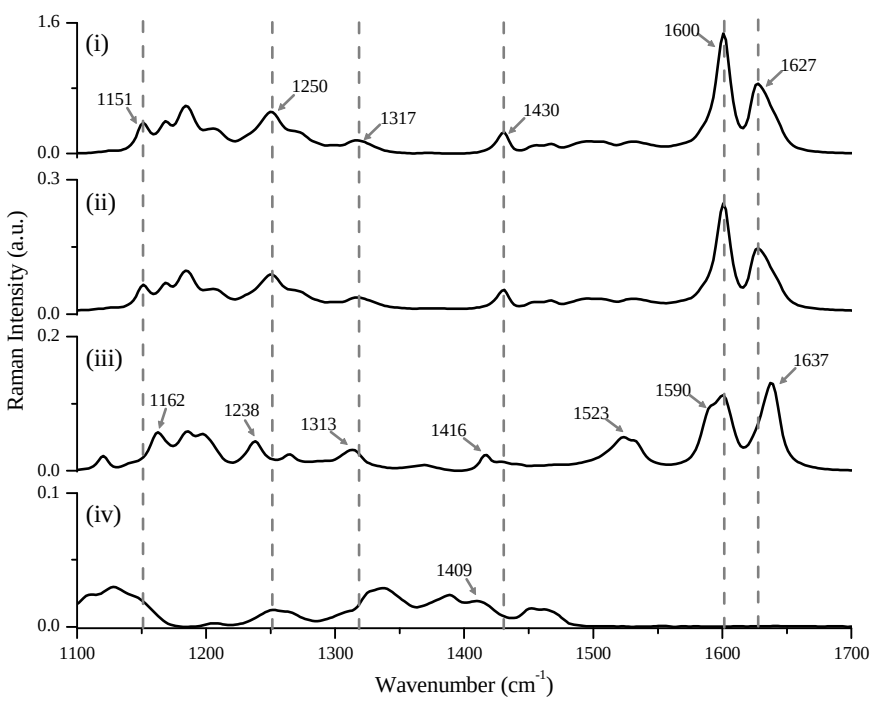
1602
1514
1587
Absorbance
Wavenumber (cm-1)

(C)


(i)
(ii)
(iii)
(iv)
1151
1250
1317
1430
1600
1627
1162
1238
1313
1416
1523
1590
1637
1409
Raman Intensity (a.u.)
Wavenumber (cm-1)

(D)

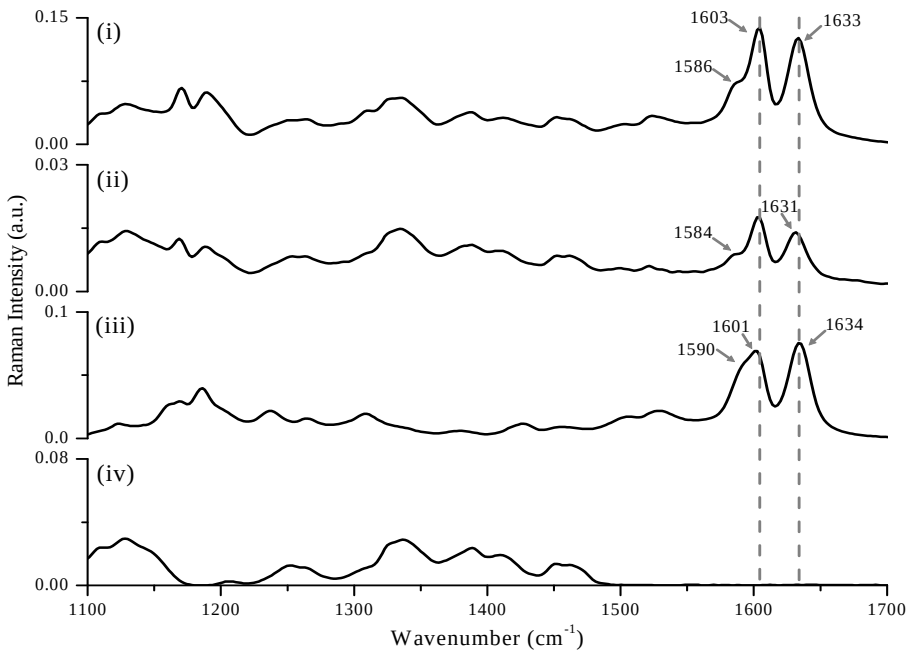
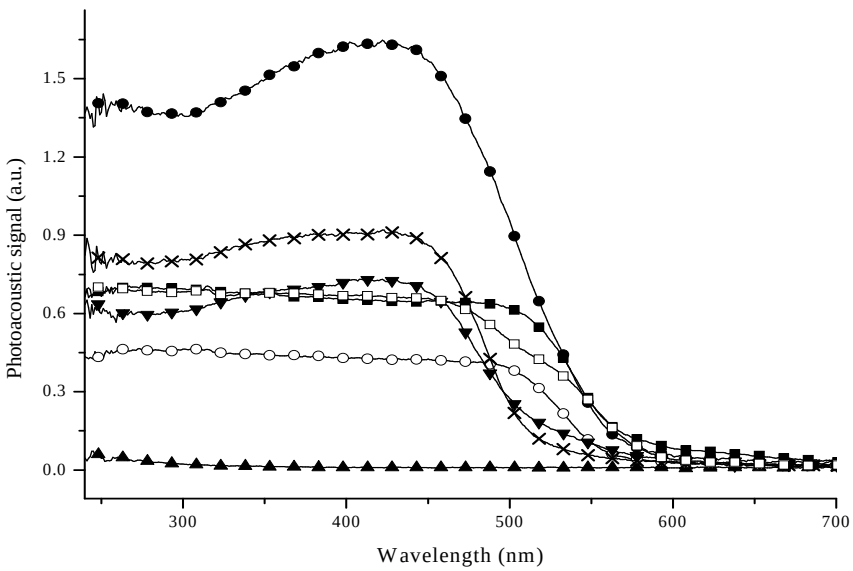


Figure 1. FT-IR spectra of (i) curcumin, (ii) simple mixture of curcumin with β-cyclodextrin in the molar ratio of 1:2, (iii) curcumin-β-cyclodextrin complex from co-precipitation and (iv) β-cyclodextrin (A); zoom in the 1400-1700 cm^{-1} region of Figure 1A of (i) curcumin and (iii) curcumin-β-cyclodextrin complex from co-precipitation spectra (B); FT-Raman spectra of (i) curcumin, (ii) simple mixture of curcumin with β-cyclodextrin in the molar ratio of 1:2, (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.

(A)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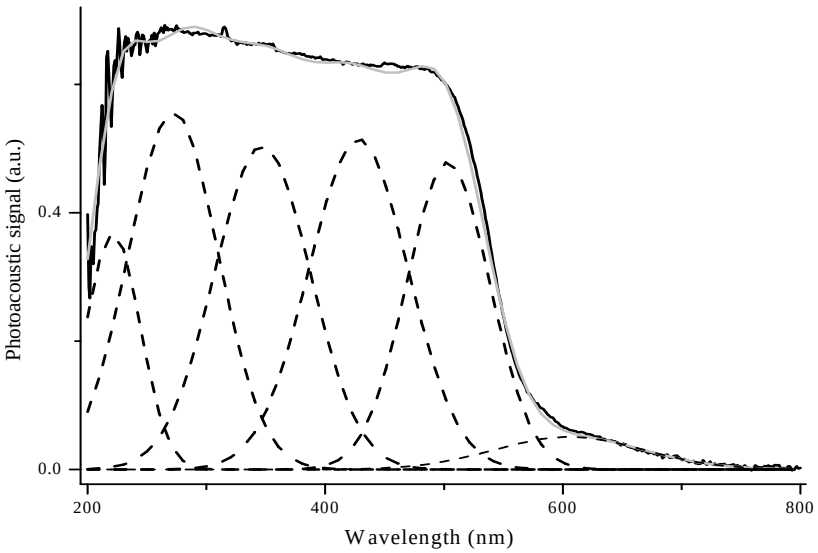
1.2
0.9
0.6
0.3
0.0
300
400
500
600
700
Wavelength (nm)

(B)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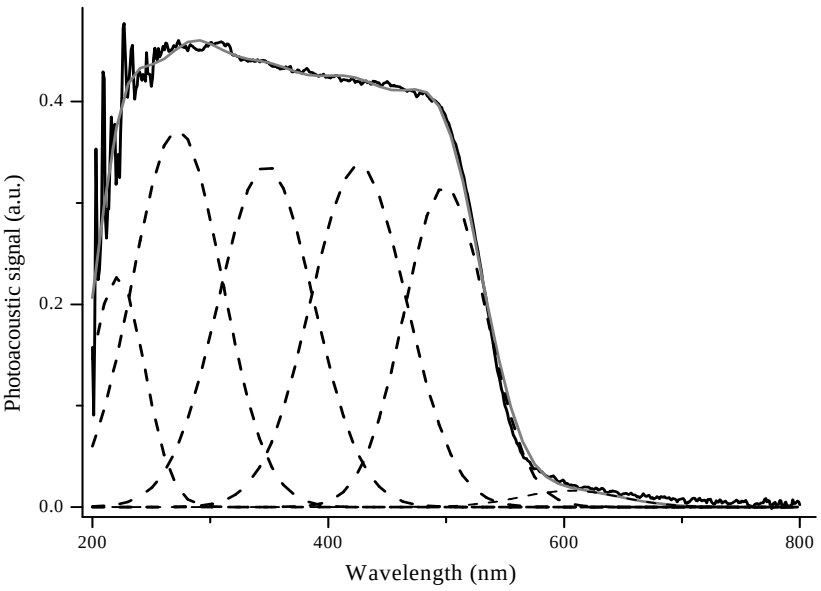
400
600
800
Wavelength (nm)

(C)


Photoacoustic signal (a.u.)
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0.0
200
400
600
800
Wavelength (nm)

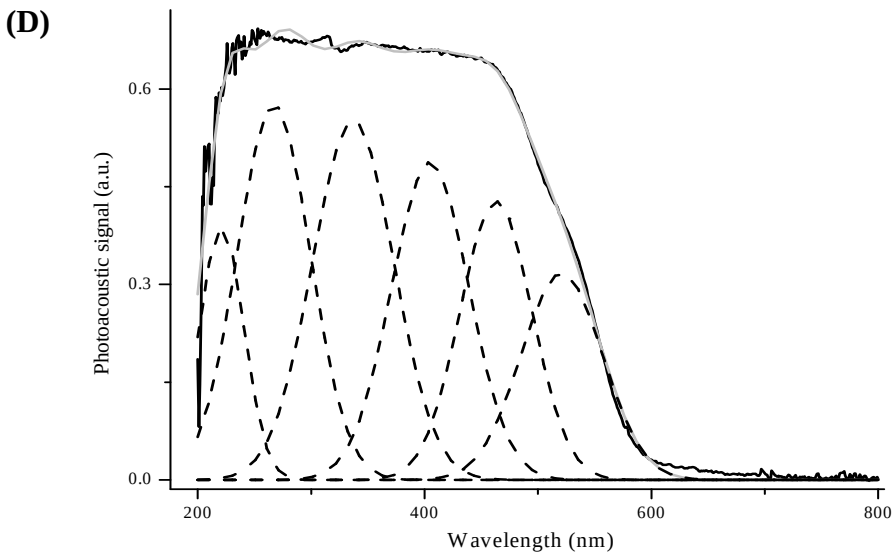

Figure 2. Optical absorption spectra of (■) curcumin, (○) the simple mixture of curcumin with β-cyclodextrin in the molar ratio of 1:2, (▲) β-cyclodextrin, (●) the curcumin-β-cyclodextrin complex from solvent evaporation, (×) the curcumin-β-cyclodextrin complex from freeze-drying with curcumin diluted 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: curcuma optical absorption spectra (B); the simple mixture of curcumin with β-cyclodextrin in the molar ratio of 1:2 optical absorption spectra (C); the curcumin-β-cyclodextrin complex from solvent evaporation optical absorption spectra (D).

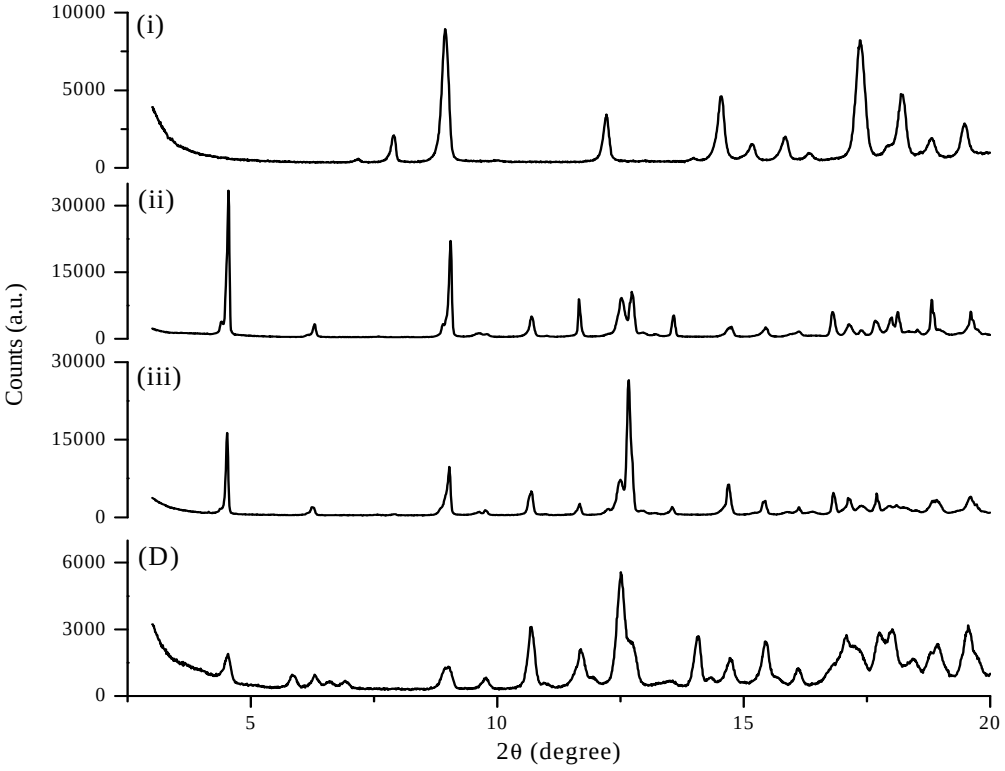

Figure 3. X-ray diffraction patterns of (i) curcumin, (ii) β-cyclodextrin, (iii) the simple mixture of curcumin with β-cyclodextrin in the molar ratio of 1:2 and (iv) the curcumin-β-cyclodextrin complex from co-precipitation.

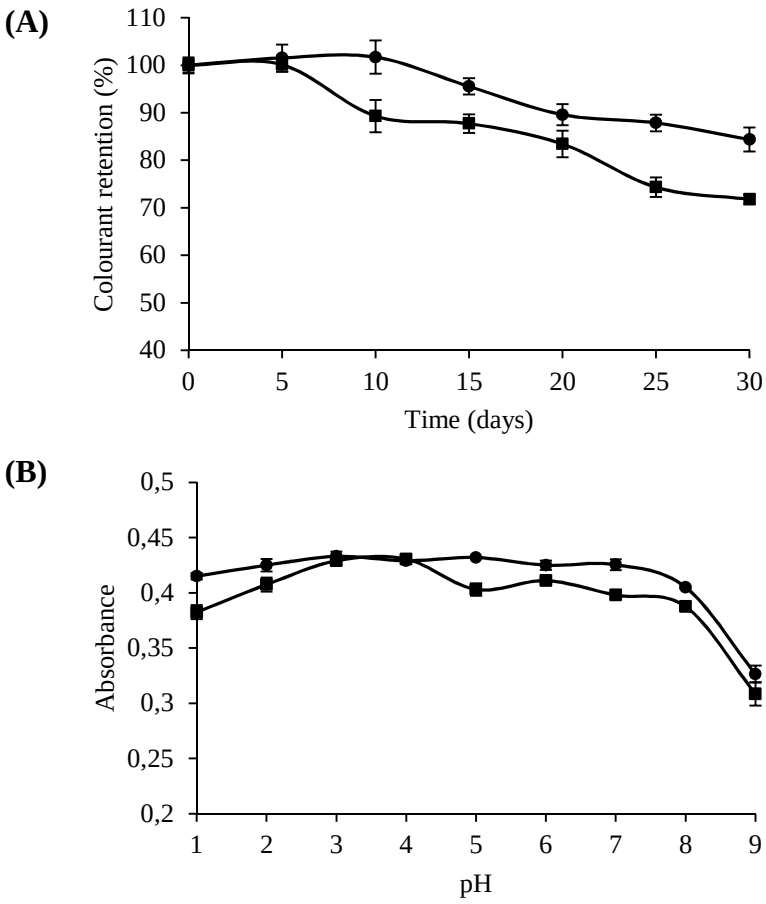

Figure 4. Curcumin retention after 30 days of exposure to natural light (A); stability in different pHs (B). Pure curcumin (■) and the curcumin-β-cyclodextrin complex (●).

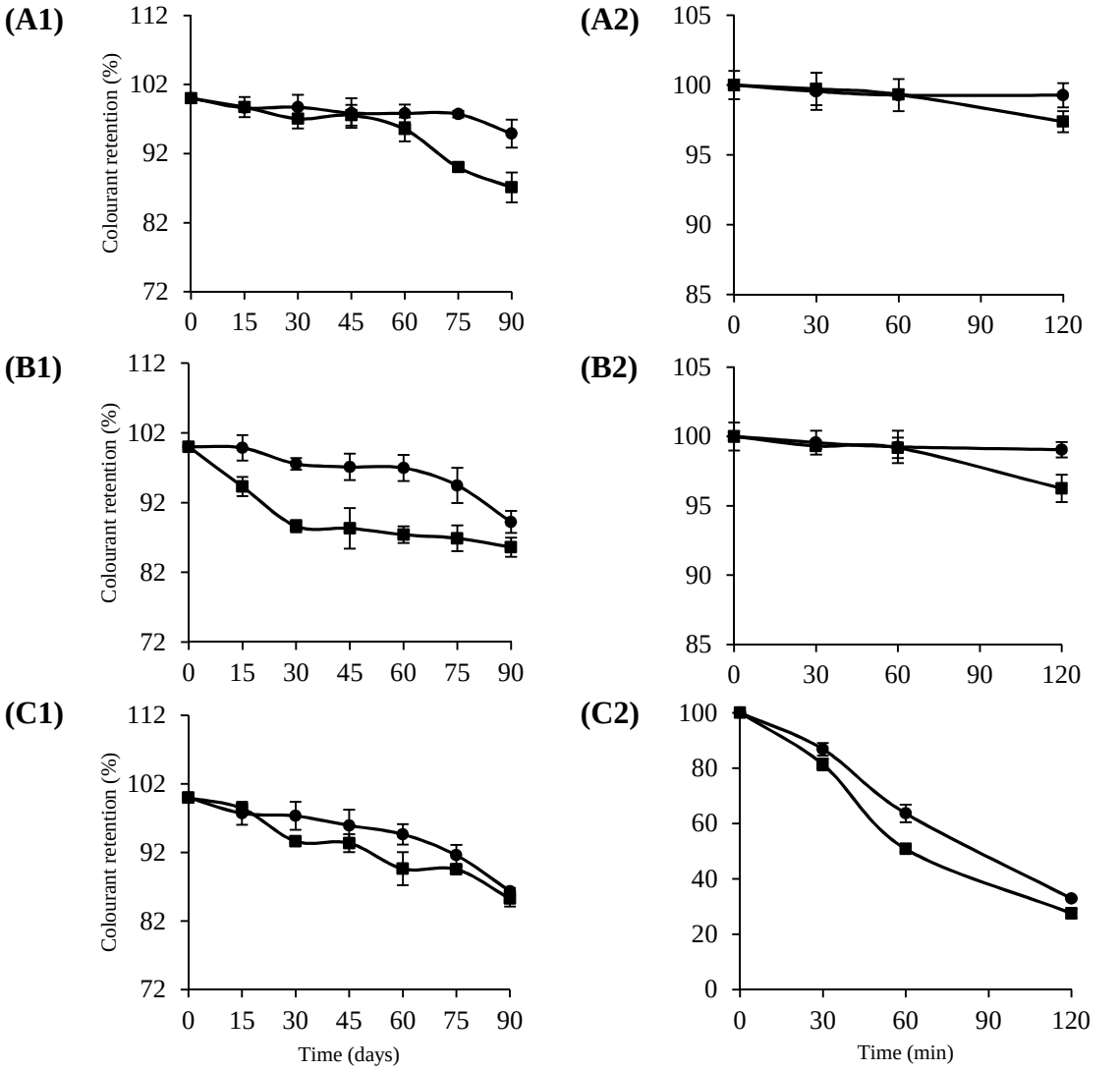

Figure 5. Curcumin retention after 90 days of storage at -15 °C (A1), 4 °C (B1) and 25 °C (C1); curcumin retention after 2 hours of an isothermal heating at 100 °C (A2), 150 °C (B2) and 200 °C (C2). Pure curcumin (■) and the curcumin-β-cyclodextrin complex (●).

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.

Table 2. The colourimetric attributes of vanilla ice creams. Formulations with 0.25 g of pure curcumin (A), 2.23 g of the curcumin-β-cyclodextrin complex (B) and 0.30 g of the curcumin-β-cyclodextrin complex (C). The values given indicate mean ± standard error.

Formulation	L*	a*	b*	Chroma	Hue (^{o})
A	90.5 ± 0.5^{a}	-6.7 ± 0.1^{a}	30.5 ± 0.7^{a}	31.3 ± 0.7^{a}	102.3 ± 0.7^{a}
B	90 ± 1^{a}	-9.4 ± 0.2^{b}	51 ± 6^{b}	52 ± 6^{b}	101 ± 1^{b}
C	91.1 ± 0.1^{a}	-8.7 ± 0.4^{c}	31 ± 2^{a}	32 ± 2^{a}	105.6 ± 0.2^{c}

[a,b] Means within the same column with different letters are significantly different ($p < 0.05$).

Table 3. Sensory attributes and buying intention of the vanilla ice creams. Formulations with 0.25 g of pure curcumin (A), 2.23 g of the curcumin-β-cyclodextrin complex (B) and 0.30 g of the curcumin-β-cyclodextrin complex (C).

Formulation	Colour	Texture	Taste	Flavor	Buying intention
A	7.70[a]	7.57[a]	7.71[a]	7.47[a]	2.53[a]
B	6.03[b]	7.46[a]	7.26[a]	7.04[a]	2.12[b]
C	7.45[a]	7.49[a]	7.59[a]	7.37[a]	2.56[a]

[a,b] Means within the same column with different letters are significantly different ($p < 0.05$).

FOOD CHEMISTRY

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