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Evaluation of stability of bixin in nanocapsules in model systems of photosensitization and heating

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ABSTRACT

Bixin is a good quencher of singlet oxygen, but is unstable at high temperatures and has low solubility in water. The aim of this study was to evaluate the stability of bixin in lipid-core nanocapsules (BIX-LNC) during photosensitization (5–25 °C) and heating (65–95 °C) in model systems of ethanol:water (2:8). The BIX-LNC were prepared by the technique of interfacial deposition of preformed polymer, with a mean diameter ($D_{4,3}$) of 195 ± 27 nm. During photosensitization in air-saturated conditions, free bixin and BIX-LNC exhibited activation energies of 7.09 and 11.48 kcal/mol, respectively, and in the absence of oxygen exhibited activation energies of 8.96 and 16.31 kcal/mol, respectively. During heating, free bixin and BIX-LNC showed activation energies of 15.06 and 23.81 kcal/mol, respectively. The activation energies for BIX-LNC were superior to those of free bixin in both the photosensitization and heating experiments, suggesting that encapsulation increased the stability of bixin.

1. Introduction

Bixin (methyl hydrogen 9′-*cis*-6, 6′-diapocarotene-6, 6′-dioate) is a carotenoid and the major colourant of annatto seeds (Mercadante, Steck, & Pfander, 1997). Bixin is a scavenger of reactive species of oxygen and nitrogen, and has ability to quench excited molecules due to its structure (formed by nine conjugated carbon double bonds, two carboxylic groups), which is a good receptor of free radicals and energy from excited molecules like singlet oxygen (Chisté et al., 2011; Dias, Pilha, Alves, Oliveira, & Munim, 2011; Rios, Mercadante, & Borsarelli, 2007).

The transfer of energy from the singlet oxygen to bixin is favourable, since oxygen in its singlet state has an energy level of 22.5 kcal/mol and bixin in its triplet state has an energy level of 18 kcal/mol. This difference in the energy levels makes bixin and efficient quencher of singlet oxygen (Rios et al., 2007). Singlet oxygen has a great importance for foods because the oxidation of some compounds, like lipids is greater in the presence of singlet oxygen than in the presence of triplet oxygen independently of temperature. Singlet oxygen is more electrophilic than triplet oxygen and reacts 1500 times faster with moieties of high electron-density, such as final carbon double bond, yielding hydroperoxides as oxidation products (Nawar, 1996).

Singlet oxygen can be generated by various ways (Yahia & Ornelas-Paz, 2010). In foods, singlet oxygen can be generated the presence of sensitizers, light and triplet oxygen. Many types of sensitizers are commonly found in foods, such as chlorophyll, pheophytins, porphyrins, riboflavin, myoglobin, and some synthetic colourants. The sensitizers can absorb light and transfer energy to triplet atmospheric oxygen to form singlet oxygen (Choe & Min, 2009). A carotenoid may act in two ways to avoid subsequent reactions caused by singlet oxygen. The first is inactivating the sensitizer in its excited state, and the second is quenching the singlet oxygen (physical and chemical quenching) (Choe & Min, 2009; Min & Boff, 2002).

Bixin is an antioxidant and a colourant used in food products. The chemical structure is responsible for its poor water-solubility, which impairs its use in some food products (Rodriguez-Amaya, 2001). Several studies have been performed to evaluate the stability of free bixin during photosensitization and heating in model

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systems (Montenegro, Rios, Mercadante, Nazareno, & Borsarelli, 2004; Rios, Borsarelli, & Mercadante, 2005; Scotter, Castle, & Appleton, 2001) and the stability of encapsulated bixin has been evaluated in aqueous solution under illumination (Barbosa, Borsarelli, & Mercadante, 2005). The photosensitized isomerization reaction of bixin with the sensitizers rose bengal and methylene blue in model system of acetonitrile:methanol (1:1, mL:mL) in N_2- and air-saturated conditions has been studied using UV–vis spectroscopy and high-performance liquid chromatography (HPLC). Singlet oxygen was formed in an laser flash photolysis experiment and its presence was verified by the measurement of phosphorescence at 1270 nm (Montenegro et al., 2004).

Parize et al. (2008) produced, characterized and evaluated the thermal stability of the urucum pigment (containing bixin) in microcapsules prepared by the technique of spray drying using chitosan as encapsulating agent in acetic acid (5 g/100 mL), lactic acid (5 g/100 mL) and citric acid (5 g/100 mL), and Barbosa et al. (2005) evaluated the light stability of spray-dried bixin encapsulated with gum arabic or maltodextrin.

Although carries out important functions to health, bixin is considered to be unstable in the presence of oxygen, heat and light. Nevertheless, some studies showed that the techniques of complexation and encapsulation decrease the degradation rates of bixin caused by light, air, ozone, oxygen and high temperature (Barbosa et al., 2005; Lyng, Passos, & Fontana, 2005; Marcolino, Zanin, Durrant, Benassi, & Matioli, 2011; Parize et al., 2008).

Nanoencapsulation is a process by which one compound is covered by another, producing particulate dispersions or solid particles with sizes ranging from 10 nm to 1 μm. Depending upon the method of preparation of nanoparticles, can be obtained nanospheres or nanocapsules. Nanocapsules are systems in which the drug is soluble in the core, confined to a cavity surrounded by a polymer membrane, while nanospheres are matrix systems in which the drug is dispersed in the structure. In general, encapsulation improves the stability, solubility and bioavailability of encapsulated species and promotes its controlled release. (Mohanraj & Chen, 2006; Ribeiro, Chua, Ichikawa, & Nakajima, 2008; Tan & Nakajima, 2005).

Taking those considerations into account, we hypothesized that nanoencapsulation could improve the stability of bixin. With this knowledge, it is possible get an indicative of stability of bixin when employed in a food as antioxidant. Thus, the aim of this was to evaluate the stability of nanoencapsulated bixin during photosensitization and heating in model system of ethanol:water (2:8, mL:mL).

2. Materials and methods

2.1. Materials

The polymer poly-ε-caprolactone (PCL) (Molecular weight = 80,000 g/mol), sorbitan monostearate (span 60) and Rose Bengal sodium salt (90% pure) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The capric/caprylic triglycerides (CCT) and polysorbate 80 (Tween 80) were obtained from Delaware (Porto Alegre, RS, Brazil). The annatto seeds were obtained from the local market in Porto Alegre, RS, Brazil. The solvents used in HPLC analysis were of chromatographic grade.

2.2. Preparation of the bixin standard

The extraction of bixin was carried out in the absence of light as previously described (Rios & Mercadante, 2004). Firstly, two solid–liquid extractions were performed using 50 mL of hexane. The seeds were separated by filtration and two solid–liquid extractions were carried out using 50 mL of methanol. The seeds were separated again and bixin was extracted twice from the seeds with 50 mL of ethyl acetate. In the present work each extraction was carried out under magnetic stirring during 15 min. The last extract (in ethyl acetate) was filtered and the solvent was removed in a rotary evaporator (Fisatom, model 801/802, São Paulo, SP, Brazil).

In a flask containing the extract solubilised in dichloromethane (5 mL), 20 mL of ethanol (99.7%) was added slowly and carefully to avoid mixing the two solvents. This recipient was placed in a cold bath for 5 min and later stored during 12 h at −18 °C to allow the crystallization of bixin. The bixin crystals formed in the bottom of the recipient were separated by filtration, simultaneously were washed with 50 mL of ethanol and dried under reduced pressure ($T < 30$ °C). The bixin standard was analysed by high performance liquid chromatography (HPLC) and was found a purity of 98.7 ± 0.20 g/100 g.

2.3. Preparation of the bixin nanocapsules

The bixin-loaded lipid-core nanocapsules (BIX-LNC) were produced by the technique of interfacial deposition of preformed polymers as previously described (Venturini et al., 2011). The organic phase was prepared by the dissolution of PCL (250 mg), CCT (400 μL), span 60 (95 mg) and bixin (0.406 mg) in 67.5 mL of acetone:ethanol (8:1, mL:mL) under magnetic stirring at 40 °C (in the absence of light). This organic phase was added into an aqueous phase (130 mL) containing Tween 80 (195 mg) and remained under stirring for 10 min. The solvents were removed and the suspension was concentrated until a final volume of 25 mL in a rotary evaporator (Fisatom, model 801/802, São Paulo, SP, Brazil).

2.4. Characterization of bixin nanocapsules

The mean diameter and the zeta potential of BIX-LNC were determined by Dynamic Light Scattering (DLS) and by electrophoretic mobility, respectively (Zetasizer® nano-ZS ZEN mod. 3600, Nanoseries, Malvern, UK). The samples were appropriately diluted with a pre-filtered (0.45 μm) MilliQ® water or 10 mM NaCl aqueous solution to determine the mean diameter and the zeta potential, respectively. The data was analysed by Dispersion Technology Software (version 4.0, Malvern Instruments, UK).

The mean diameter of BIX-LNC was also determined by laser diffraction (Mastersizer 2000® 5.54, Malvern Instruments, UK), using water as dispersant. The refractive indexes used for the PCL and for water were of 1.590 and 1.330, respectively. The data was analysed by Mastersizer 2000 software programme (version 5.54, Malvern Instruments, UK). The equipments Zetasizer nano ZS® and Mastersizer 2000® determine particle sizes ranging from 0.003 to 10 μm and from 0.02 to 2000 μm, respectively. The use of both equipments allowed a wide range of particle size determination.

The viscosity of BIX-LNC suspension was determined at 25 °C using a rotational viscometer (Model DV-II + Pro, spindle LV2, Brookfield Engineering, USA). The data were analysed by Brookfield Rheocalc 32 software. The pH of the BIX-LNC suspension was determined at 25 °C using a potentiometer (Model DM-22, Digimed, Brazil).

The total concentration of bixin was determined through the extraction of bixin from the BIX-LNC suspension. This method consisted in the extraction from an aliquot of 250 μL of BIX-LNC suspension with acetonitrile (4.75 mL). This extract was sonicated in ultrasound (30 min), centrifuged (15 min at 2,820 × g) and the supernatant was injected in the HPLC without dilution. The bixin content in the aqueous phase of the BIX-LNC suspension was determined through the direct injection of the aqueous phase which was obtained after the ultrafiltration/centrifugation of an

aliquot of BIX-LNC suspension (400 μL) using a Ultrafree-MC® (10,000 Molecular Weight, Millipore, Bedford, USA) under centrifugation (15 min at 1,690 × *g*). The encapsulation efficiency (%) was determined by dividing the difference between the total concentration of bixin and the concentration of bixin in the aqueous phase by the total concentration and multiplying the results by 100 (Venturini et al., 2011).

2.5. Photosensitization in model system

In the present study, the photosensitization solution containing the BIX-LNC was prepared diluting the BIX-LNC suspension and an aliquot of a rose bengal solution (quantified previously by spectrophotometer UV–Visible Agilent 8453, Santa Clara, CA, USA) in ethanol:water (2:8, mL:mL). The bixin and the rose bengal concentrations applied in this study were of 8 μmol/L (3.16 μg/mL) and 10 μmol/L, respectively. Aliquots of 10 mL of the photosensitization solution containing BIX-LNC and the sensitizer rose bengal was illuminated with a 150 W filament lamp (36,000 lux) coupled to an orange acrylic cutoff filter to exclusively excite the sensitizer (at wavelengths above 520 nm). The experiment of photosensitization was performed in model system of ethanol:water (2:8, mL:mL) to allow the comparison with free bixin, which is insoluble in water.

The photosensitization experiments were carried out in three temperatures (5, 15 and 25 °C). In each temperature, two different conditions were tested. The first was performed in the absence of oxygen injecting N_2 (99.99% purity) to the photosensitization cell and the second condition was carried out in an air-saturated condition.

In the experiment of photosensitization, the filtered light excites rose bengal and energy is transferred to oxygen (atmospheric oxygen) generating singlet oxygen. In the presence of oxygen (air-saturated condition), both sensitizer (rose bengal) and singlet oxygen simultaneously participates in the loss of bixin, whereas in the absence of oxygen (N_2-saturated condition), only the sensitizer in its excited state is responsible for the loss of bixin (Foote, Chang, & Denny, 1970).

A preliminary experiment of photosensitization involving free bixin (bixin standard) (8 μmol/L = 3.16 μg/mL) was performed under the same conditions of BIX-LNC in model system of ethanol:water (2:8, mL:mL) at 25 °C. The content of bixin was determined periodically by HPLC (0, 15, 30, 45, 60, 90, 120, 180, 240 and 300 min. In the present study we observed that the consumption of bixin occurred only in the presence of light (filtered light) and no changes in the bixin content was verified in the absence of light even in air-saturated conditions.

The concentration data were used to determinate the kinetics parameters using the Origin Pro 8.0 software (Origin lab Co., MA, USA).

2.6. Heating in model system

For the experiments of heating, a solution of nanocapsules was prepared through the dilution of an aliquot of the BIX-LNC suspension in ethanol:water (2:8, mL:mL) until a bixin concentration of 8 μmol/L (3.16 μg/mL). Several aliquots (2 mL) of this solution were heated in glass test tubes with screw caps (20 mm of diameter and wall thickness of 1 mm) in a heating bath at 65, 80 and 95 °C. After the heating, each sample was cooled in an ice bath. The heating of free bixin (non-encapsulated) (8 μmol/L) was performed under the same conditions of BIX-LNC in model system of ethanol:water (2:8, mL:mL) in the dark. The bixin content was evaluated periodically by HPLC in the following periods: 0, 15, 30, 45, 60, 90, 120, 180, 240 and 300 min. The bixin content data were used to determinate the kinetics parameters by the Origin Pro 8.0 software (Origin lab Co., MA, USA).

2.7. Analysis of high performance liquid chromatography (HPLC)

Analyses were carried out according to Montenegro et al. (2004) using an HPLC system that consisted of an online degasser, a quaternary pump and an automatic injector (Agilent series 1100, Santa Clara, CA, USA) and a C18 Spherisorb ODS-2 column (150 × 4.6 mm i.d.; 3 μm particle size). The samples were eluted isocratically using as the mobile phase acetonitrile/acetic acid (2%)/dichloromethane (63:35:2, mL:mL:mL) at a flow rate of 1 mL/min at 25 °C and bixin was detected at 470 nm. Data acquisition and processing were performed using the CHEMSTATION® software programme.

All of the solvents used in the HPLC separation were of chromatographic grade and previously filtered through a Millipore vacuum filtration system using a 0.22 μm membrane for organic solvents (Millipore, SP, Brazil). Before being injected into the chromatograph all samples were filtered using a modified PTFE membrane for aqueous and organic solvents with pore diameter of 0.45 μm (Millipore, SP, Brazil) and each injection was carried out in duplicate.

For the quantification of bixin, a standard curve with a determination coefficient (R^2) greater than 0.99 was used. This standard curve was obtained plotting the peak areas (from the HPLC) of solutions containing different concentrations of bixin (from 1.37 to 80.16 μg/mL). Each solution was quantified previously by spectrophotometry (UV–Visible Agilent 8453, Santa Clara, CA, USA) at 470 nm using an absorptivity coefficient of 2826 in chloroform. The limits of detection (LOD) and quantification (LOQ) were determined as described previously by Long and Winefordner (1983) and were of 0.231 μg/mL and 0.235 μg/mL, respectively.

3. Results and discussion

3.1. Characterization of bixin nanocapsules

The bixin nanocapsules (BIX-LNC) produced in this work exhibited a monomodal particle-size distribution with all particles with diameters smaller than 1 μm, a mean diameter of 190 ± 9, a polydispersity index of 0.1 ± 0.03, a volume-weighted mean diameter ($D_{4,3}$) of 195 ± 27 nm, a surface-weighted mean diameter ($D_{3,2}$) of 138 ± 13 nm and a span value of 1.4 ± 0.1. The BIX-LNC suspension was produced with a bixin concentration of 16.23 μg/mL (8 μmol), and showed a zeta potential of −14.45 ± 0.92 mV, pH of 5.9 ± 0.70, encapsulation efficiency of approximately 100% and viscosity of 11.4 ± 0.24 mPa s (Lobato et al., 2013).

Similar results were found by other authors that used this same formulation for the production of nanocapsules or nanospheres (Jäger et al., 2009; Pohlmann, Weiss, Mertins, Silveira, & Guterres, 2002; Paese et al., 2009). Paese et al. (2009) produced benzophenone-3-loaded lipid-core nanocapsules with a mean diameter of 247 ± 4 nm, a polydispersity index lower than 0.2, a pH of 6.56 ± 0.09 and a zeta potential of −9.5 ± 1.0 mV. In other study, indomethacin-loaded lipid-core nanocapsules exhibited a mean pH of 4.2 ± 0.1 (Pohlmann et al., 2002). The indomethacin ethyl ester nanocapsules were produced with zeta potentials ranging from −8.6 ± 0.1 to −12.7 ± 2.5 mV (Jäger et al., 2009).

3.2. Photosensitization in model system

The nanoparticle size control is a parameter that must be ensured during storage, since one form to verify if a nanoparticle formulation is physically stable is the periodic determination of the mean diameter (Wu, Zhang, & Watanabe, 2011). In a preliminary

experiment, the BIX-LNC suspension was diluted in ethanol:water (2:8, mL:mL) at 25 °C and the mean diameters of the BIX-LNC were determined periodically (0, 15, 30, 60, 120, 180, 240 and 300 min) (Fig. 1a). This procedure was applied to verify if the nanocapsules would be physically stable during the photosensitization experiments.

Immediately after preparation, the BIX-LNC exhibited a monomodal particle size distribution with a mean particle diameter of 199.9 ± 1.8 nm and a polydispersity index of 0.12 ± 0.01 (Fig. 1b). The PDI values ranging from 0.1 to 0.25 indicates a narrow size distribution while a PDI greater than 0.5 is related to a broad distribution (Wu et al., 2011). No significant changes ($p < 0.05$) were verified in the nanocapsules mean diameter and polydispersity index values during 300 min at 25 °C, suggesting that BIX-LNC would be physically stable in the ethanol:water (2:8, mL:mL) solution during photosensitization.

The rate of bixin loss (free and in BIX-LNC) increased with the change of temperature of the system from 5 °C to 25 °C in the presence and in the absence of oxygen (N_2-saturated conditions). The presence of oxygen (air-saturated condition) in the photosensitization system increased the rate of bixin loss (free and in BIX-LNC), since in the first case (air-saturated conditions) both singlet oxygen and the sensitizer rose bengal acted in the degradation of bixin (Fig. 2).

The nanocapsule structure allowed bixin to quench the sensitizer rose bengal and singlet oxygen. In all of the tested conditions bixin degradation followed a first order reaction kinetics with a mean coefficient of correlation R^2 above 0.99. In other study, during photosensitization in a model system of acetonitrile:methanol (1:1), the consumption of bixin is accompanied by an increase in the levels of all-*trans*-bixin, which is considered to be the main product formed (Montenegro et al., 2004). In the present work, the rate constants (k) for bixin loss during photosensitization in ethanol:water (2:8) was higher for free bixin than for bixin in the BIX-LNC in both air-saturated and N_2-saturated solutions in the same temperatures (Table 1). This result might be related to the ability of the nanocapsule to release slowly its core, which results in "free" bixin in the aqueous media (Jäger et al., 2009). Another explanation for the lower rate constants of BIX-LNC may be related to the permeability of singlet oxygen in the nanocapsule structure. These results indicate that the quenching activity of bixin is prolonged by nanoencapsulation.

In an experiment of photosensitization of bixin (8–76 μmol/L) in model system of methanol:acetonitrile (1:1, mL:mL) using rose bengal (10 μmol/L) as the sensitizer at 5, 15 and 20 °C were observed rate constants (k) of 2.3×10^{-4}, 3.00×10^{-4} and 3.7×10^{-4} s^{-1}, respectively. The highest rate constant value (4.2×10^{-3} s^{-1}) was found for bixin (8 μmol/L) at 20 °C in the air-saturated condition (Montenegro et al., 2004). The discrepancy between the rate constants values found by Montenegro et al. for bixin at the same concentration (8 μmol/L) and the present study may be explained by the type and the concentration of the organic solvents used in the model system. The activation energies for bixin loss in the present investigation revealed that under all of the conditions, the BIX-LNC exhibited a higher activation energy than did the free bixin, indicating that encapsulation increases bixin stability during photosensitization in model system of ethanol:water (2:8, mL:mL).

3.3. Heating in model system

A preliminary experiment of heating free bixin and BIX-LNC at 40 °C during 120 min was conducted to verify a possible loss of bixin. However, we observed that bixin concentration was constant during the experiment. Based on these results, the tests of heating were carried out at 65, 80 and 95 °C during 120 min (Fig. 3).

In all conditions, the rate of bixin loss (free or in BIX-LNC) increased with the increase of temperature and followed first-order kinetics with a correlation coefficient R^2 greater than 0.99. Barbosa et al. (2005) found greater stability for encapsulated bixin by spray-drying with gum arabic or maltodextrin compared to free bixin, and free bixin loss in the presence of light followed a first-order decay. However, the kinetic behaviour of the photodegradation of bixin encapsulated was formed of two sequential first-order decays, where the first decay (first period) was faster than the second. The authors found that the kinetic behaviour of the fast decay for encapsulated bixin was similar to the kinetic behaviour of free bixin under the same conditions, indicating that the fast decay resulted from the degradation of bixin located outside of microcapsules. Rios, Borsarelli, & Mercadante (2005) verified that the degradation of bixin (76 μmol) in a model system of ethanol:water (2:8, mL:mL) during heating at 70, 77, 84, 98, and 125 °C in the dark did not fit to the first-order rate law (exponential fitting). Although, a good fitting was obtained using a biexponential model due to the presence of a reversible step in the bixin degradation mechanism.

In the present work, during heating, the loss of the free bixin occurred more rapidly than did that of the bixin in the BIX-LNC, as demonstrated by the higher values of rate constants and activation energies (Table 2). Scotter et al. (2001) found rate constants (k) of 0.68×10^{-5}, 1.08×10^{-5} and 2.08×10^{-5} s^{-1} in model systems of methanol, ethanol and propanol at 64.6 °C, 78.3 °C and 97.2 °C,

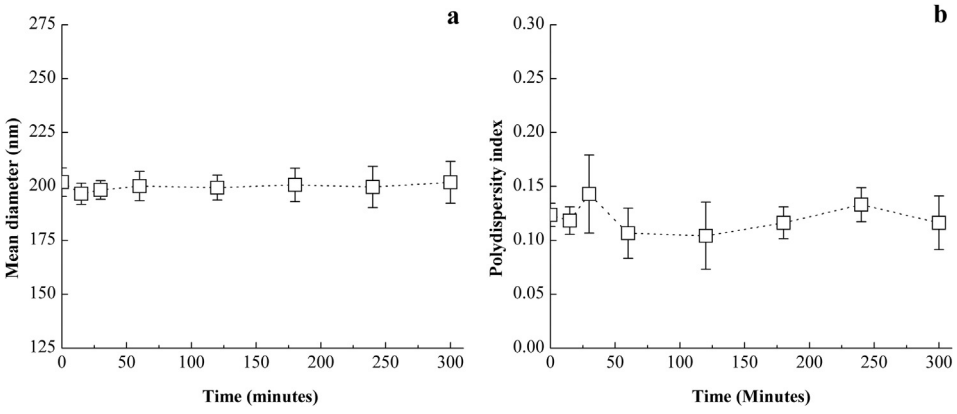

Fig. 1. Mean diameters (a) and polydispersity indexes (b) of BIX-LNC in the model system of ethanol:water (2:8, mL:mL) throughout 300 min at 25 °C.

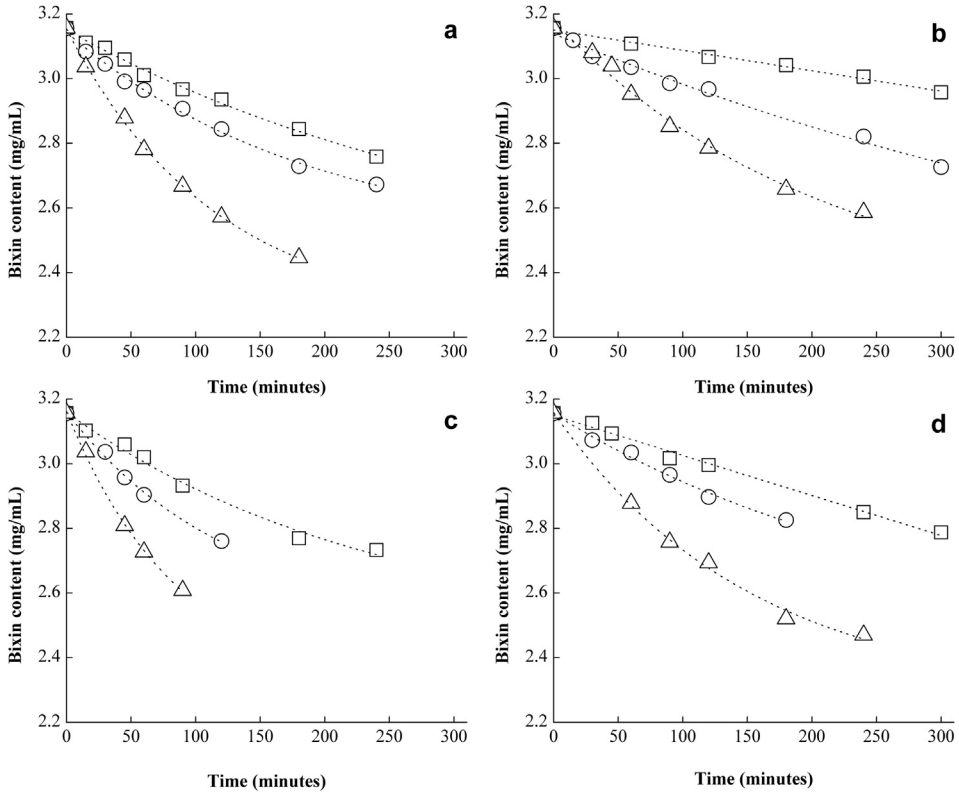

Fig. 2. Bixin concentration during photosensitization of free bixin (a) and BIX-LNC (b) in a N_2-saturated solution, respectively, and bixin concentration during photosensitization of free bixin (c) or BIX-LNC (d), respectively, in an air-saturated solution at 5 °C (□), 15 °C (○) and 25 °C (△) in model system of ethanol:water (2:8, mL:mL).

respectively, in a study of the thermal degradation of *cis*-bixin. Scotter, Castle and Appleton observed that the degradation of *cis*-bixin followed a first order kinetics with an activation energy of 8.53 kcal/mol and the main degradation products formed during heating in ethanol were di-*cis*-bixin, *trans*-bixin and 4,8-dimethyl-tetradecahexaene-dioic acid.

During the heating of the bixin in model system of ethanol:-water (2:8, mL:mL) at 70, 98 and 125 °C, the rate constants (k) found were of 1.67×10^{-6}, 2.33×10^{-5} and 2.5×10^{-4} s^{-1}, respectively. Among the degradation compounds, all-*trans*-bixin was formed only at 125 °C with an energy of 24 kcal/mol (Rios, Borsarelli, & Mercadante, 2005).

In other study involving bixin microcapsules produced by spray-drying, the lower degradation rate was attributed to the micro-encapsulated bixin which was approximately 10 times more stable than free bixin. The instability of bixin in two of the microcapsule formulations was associated to their low encapsulation efficiencies, which means more "free" bixin in the media. (Barbosa et al., 2005). In the present study was obtained an encapsulation efficiency of 100 ± 0.02%, which may have improved the bixin stability.

Encapsulation also improved the thermal stability of other carotenoids. Sáiz-Abajo, González-Ferrero, Moreno-Ruiz, Romo-Hualde, and González-Navarro (2013) investigated the incorporation of β-carotene in casein micelles and its thermal stability and observed that casein micelles protected β-carotene against heat degradation not only during long periods at 80 °C but also when two heat industrial treatments such as pasteurisation and sterilisation were applied. Approximately 84% of the total β-carotene concentration in control samples (free β-carotene) was lost after 8 h of heating, whereas in casein micelles the β-carotene loss was around 31%. The authors also verified that after the baking process of cookies at 180 °C, the degradation was lower for encapsulated β-carotene than for free β-carotene added to the cookies. The authors attributed the higher stability of the micelles due to intra and intermolecular hydrophobic interactions between casein and β-carotene.

Tachaprutinun, Udomsup, Luadthong, Wanichwecharungruang (2009) investigated if encapsulation with PCLC (poly (ethylene oxide)-4-methoxycinnamoylphthaloyl-chitosan) could improve the thermal stability of astaxanthin. When astaxanthin solution (in ethanol) was heated to 70 °C for two hours in ethanol, most molecules were degraded. However, for encapsulated astaxanthin tested in same conditions no significant changes were observed after heating, indicating that thermal degradation of astaxanthin can be prevented by encapsulation.

Other studies have reported that encapsulation improved the stability of other carotenoids during storage. Tan and Nakajima (2005) reported that β-carotene remained stable for 3 months at 4 °C (in the absence of light) in a nanodispersion produced with Tween 20 as the emulsifier. In other study, Ribeiro et al. (2008) observed that β-carotene in nanoparticles produced with poly-D,L lactic acid and poly-D,L-lactic-co-glycolic acid was stable for 5 months of storage at 4 °C. Ribeiro et al. (2008) and Qian, Decker, Xiao, and McClements (2012) added antioxidants (ascorbic acid, vitamin E acetate, coenzyme Q10 and EDTA) to decrease the

Table 1
Rate constants (k) and activation energies (Ea) for the degradation of free and encapsulated bixin (BIX-LNC) during photosensitization in model system of ethanol:water (2:8, mL:mL).

Sample	Condition	Rate constants k (s^{-1})			Ea (kcal/mol)
		5 °C	15 °C	25 °C	
Free bixin	Air-saturated	8.52×10^{-5}	1.33×10^{-4}	2.02×10^{-4}	7.09
	N_2-Saturated	4.88×10^{-5}	8.78×10^{-5}	1.45×10^{-4}	8.96
BIX-LNC	Air-saturated	2.71×10^{-5}	5.86×10^{-5}	1.09×10^{-4}	11.48
	N_2-Saturated	1.11×10^{-5}	2.81×10^{-5}	8.04×10^{-5}	16.31

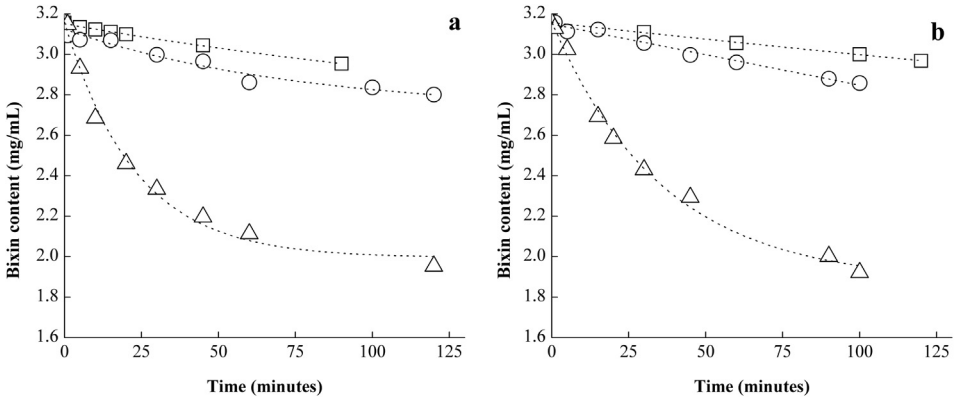

Fig. 3. Bixin concentration during heating of free bixin (a) and BIX-LNC (b), respectively, at 65 °C (□), 80 °C (○) and 95 °C (△) in model system of ethanol:water (2:8, mL:mL).

Table 2
Rate constants (k) and activation energy (Ea) for bixin loss during heating in an ethanol:water (2:8) solution of free bixin or BIX-LNC.

Sample	Rate constants k (s^{-1})			Ea (kcal/mol)
	65 °C	80 °C	95 °C	
Free bixin	1.16×10^{-4}	2.85×10^{-4}	7.25×10^{-4}	15.06
BIX-LNC	1.94×10^{-5}	7.86×10^{-5}	3.51×10^{-4}	23.81

degradation rate of β-carotene in nanodispersions and nanoemulsions.

Although the addition of antioxidants could be considered an alternative to decrease the degradation of bixin, in all conditions tested in the present study, encapsulation proved to be a technique to increase the stability of bixin in the model system of ethanol:-water (2:8, mL:mL) during photosensitization and during heating. The results obtained in this study extend the knowledge about the stabilization promoted by encapsulation during photosensitization and heating. Nanoencapsulation may increase the applications of bixin in the enrichment of foods or in other kinds of products in which its antioxidants activities are needed. Moreover, the characteristics of the formulation of this study can be very useful to apply bixin in pharmaceutical studies involving the protection by an antioxidant activity.

4. Conclusions

In this study, with interfacial deposition technique preformed polymer was possible to produce bixin nanocapsules with good characteristics of particle size distribution and high stability in ethanolic solution during 5 h storage at 25° C. The nanoencapsulation also increased the stability of bixin in both experiments of photosensitization and heating in model systems of ethanol:water (2:8, mL:mL), since in all conditions, nanoencapsulated bixin exhibited higher values of energy of activation than free bixin. Moreover, this study gives indication that nanoencapsulation can prolong the ability of bixin to quench excited sensitizer and singlet oxygen.

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