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Production of solid lipid microparticles loaded with lycopene by spray chilling: Structural characteristics of particles and lycopene stability

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ABSTRACT

Solid lipid microparticles (SLM) loaded with lycopene/sunflower oil solution have been produced using the spray chilling technique and a shortening as carrier. Six treatments were formulated and evaluated with regard to size distribution, morphology, Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction. In addition, the stability of lycopene into SLM was evaluated by periodic quantification and instrumental color parameters measurements at different storage conditions. The microparticles produced in this study were spherical and no distinct bonds were detected by FTIR in lycopene/sunflower oil solution and microparticles. Moreover, X-ray diffraction analyses revealed the presence of polymorphic form β′ in the carrier (shortening) and in the microparticles. Stability studies indicated that the best conditions to delay the degradation of encapsulated lycopene was achieved with the formulation containing gum Arabic and storage under refrigeration and vacuum. Results obtained in the present study show that lycopene was stable after incorporation into SLM, encouraging future works to evaluate the bioavailability of encapsulated lycopene in both animal and human models.

1. Introduction

Lycopene is an acyclic carotenoid with 11 conjugated double bonds, which is responsible for the red color of tomatoes, guavas and watermelons. According to Wang (2012), the antioxidant capacity of lycopene may reduce the risk of several diseases. However, due to the high number of double bonds, lycopene is susceptible to oxidation and isomerization during processing steps and storage, particularly when stored in the presence of oxygen (Matioli and Rodriguez-Amaya, 2002). In this context, microencapsulation techniques such as spray drying, emulsion, molecular inclusion, complex coacervation and microemulsion have been used to overcome this drawback (Matioli and Rodriguez-Amaya, 2002, 2003; Shu et al., 2006; Blanch et al., 2007; Chiu et al., 2007; Nunes and Mercadante, 2007; Rocha et al., 2012; Silva et al., 2012; Chen et al., 2014).

One remarkable characteristic of carotenoids is the great solubility in apolar solvents such as edible fats and oils, and low solubility in water (Belitz and Grosh, 1999). However, this feature can interfere with lycopene administration, leading to extremely low bioavailability (Chen et al., 2014). In order

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to improve oral bioavailability of lycopene, Faisal et al. (2010) suggested the use of a lipid-based formulation. In this context, the application of spray chilling to produce solid lipid microparticles (SLM) loaded with lycopene using fat as carrier could be a good choice due to several aspects: (1) protection of the carotenoid; (2) improvement of lycopene bioavailability; (3) low interaction of lycopene with other compounds when it is applied in a food; (4) specific release in the intestine, during fat digestion.

Spray chilling is a process to produce microparticles in which a mixture of the molten carrier and the active ingredient is sprayed into a cold chamber using an atomizing nozzle. When the droplets meet the cold environment of the chamber, microparticles are formed by fat solidification. According to Okuro et al. (2013a), spray chilling is a convenient technique for microparticles production because it is a low-cost continuous process that is easy to scale up and does not require solvents. In addition, spray chilling does not require the application of high temperatures that are found in spray drying, which should be considered when thermolabile ingredients (*e.g.* lycopene) need to be encapsulated.

Thus, in this study, SLM loaded with lycopene were produced by spray chilling using a shortening as carrier, and the microparticles were characterized with regard to structure and lycopene stability.

2. Materials and methods

2.1. Materials

The carrier used to produce the microparticles was a shortening composed of hydrogenated and interesterified cottonseed, soy and palm oils (Tri-HS-48) supplied by Triângulo Alimentos (Itápolis, Brazil), with melting point at 51 °C. This carrier was chosen due to the availability in actual market, and because it is commonly used in food products. Gum Arabic (Dinâmica Química Contemporânea Ltda., Diadema, Brazil) and carboxymethylcellulose (CMC, Fagron do Brasil Farmacêutica Ltda., São Paulo, Brazil) were also used to produce the microparticles. The core or active material used was Redivivo®, a commercial ready to use lycopene dispersed in sunflower oil, containing 10% of lycopene (DSM, São Paulo, Brazil). The fatty acid composition of the shortening used as carrier was evaluated by FAME (fatty acid methyl esters) gas chromatography, according to the official methods AOCS Ce 2-66 and Ce 1-62 (AOCS, 1998), using a Shimadzu 2010 AF Gas Chromatograph (Kyoto, Japan) coupled with an automatic injector (AOC20i, Shimadzu, Kyoto, Japan) and a flame ionization detector. The analysis was performed in triplicate and the carrier composition is presented in Table 1.

2.2. Production of microparticles

Solid lipid microparticles were produced using the spray chilling technique as described by Salvim et al. (2015), with modifications. Lycopene was incorporated into the shortening previously molten at 60 °C and atomized using an spray chiller (Model MSD 1.0, Labmaq do Brasil, Ribeirão Preto, Brazil) in a chamber kept at 13 °C by an air stream system, with an 1.2 mm nozzle, 1.0 kgf/cm^2 air pressure and 40 ml/min of feed flow (controlled by peristaltic pump). Six formulations were used to produce SLM, as described in Table 2. The particles were produced three times, and all further analyses were performed in triplicate.

Table 1 – Fatty acid composition of the shortening used as carrier in this study. The analyses were performed by gas chromatography coupled with flame ionization detector, and results represent the ratio of each fatty acid in the carrier.

Fatty acid	Composition (g/100 g)
Lauric (C12:0)	0.46 ± 0.00
Myristic (C14:0)	0.83 ± 0.01
Palmitic (C16:0)	30.9 ± 0.7
Stearic (C18:0)	35.0 ± 0.7
Oleic (C18:1)	28.0 ± 0.3
Linoleic (C18:2)	4.7 ± 0.1
Eicosatrienoic (C20:3)	0.19 ± 0.00

2.3. Lycopene quantification

Lycopene was quantified by spectrophotometric methods, as described by Rodriguez-Amaya (2001). For that, lycopene was extracted from 10 mg of microparticles added to 10 ml of petroleum ether, and analyzed at 470 nm in a spectrophotometer (Hach, DR 2800, Loveland, USA). The quantification of lycopene was determined according to the following Eq. (1):

$$\text{Lycopene concentration} = \frac{A \times V \times 10^6}{A^{1\%}(\varepsilon) \times m \times 100} \quad (1)$$

In the formula: A is the absorbance, V is the final volume (ml), $A^{1\%}$ is the lycopene absorption coefficient in petroleum ether (3450 cm^{-1}), and m is the sample mass (g).

Similarly, the degradation of the encapsulated material was determined with the following equation (2):

$$\text{Lycopene degradation} = \left(\frac{F}{I} \times 100\right) - 100 \quad (2)$$

In the formula: I is the concentration of lycopene at the first day (mg/kg) and F is the concentration of lycopene after 3, 30, 60 and 90 days (mg/kg).

2.4. Microparticles characterization

2.4.1. Size distribution and volume weighted mean diameter (D4,3)

Size distribution and volume weighted mean diameter (D4,3) of SLM produced in this study were evaluated periodically using a laser diffraction particle analyzer (Shimadzu Sald-201V, Kyoto, Japan). Before analyses, SLM were added to ethanol (Synth, Diadema, Brazil) with rapid application of ultra-sound for better dispersion of the material.

2.4.2. Morphology

The morphology of SLM was evaluated by scanning electron microscopy (SEM) using the TM3000 Tabletop Microscope (Hitachi, Tokyo, Japan) along with the program TM3000. Microparticles were arranged in a double sided carbon tape (Ted Pella Inc., Redding, USA) and fixed in aluminum stubs. Images were acquired at 5 kV of acceleration and 1750 mA.

2.4.3. Fourier transform infrared (FTIR) spectroscopy

The chemical structures of samples were evaluated using the Perkin Elmer FT-IR Spectrometer (Massachusetts, USA) with the Spectrum One software (version 5.3.1). Sixteen scans