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Stability of curcumin encapsulated in solid lipid microparticles incorporated in cold-set emulsion filled gels of soy protein isolate and xanthan gum

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ABSTRACT

The objective of this study was to investigate the feasibility of producing cold-set emulsion filled gels (EFG), using soy protein isolate (SPI) and xanthan gum (XG) and incorporating curcumin-loaded solid lipid microparticles (SLM). For this purpose, the formulation G_{XG} (15%, w/v SPI, 0.1%, w/v XG and 5 mM $CaCl_2$) was selected for the production of EFG. A comparative study on the rheological and microstructural properties of non-filled gels and EFG revealed that SLM stabilized with Tween 80-Span 80 behaved as active fillers in the gel matrix, increasing the Young's modulus from 1.1 to 2.3 kPa, and also increasing the values of storage and loss moduli. The incorporation of SLM also affected the microstructural organization of the systems. Whereas unfilled gels presented a microstructural organization similar to that of interpenetrated networks, EFG exhibited a microstructure with clear phase separation. The stability of encapsulated curcumin in EFG was monitored using a colorimetric test and it was confirmed that the bioactive component showed a high stability for 15 days. After that period, the color started to change, indicating a decrease in curcumin concentration. The instability of curcumin was probably related to structural alterations of the EFG, which led to decreases of hardness after 7 days of storage at 10 °C, and to the collapse of the structures after 30 days. Although formulation improvements are required, the results indicate that the encapsulation of curcumin in SLM incorporated in EFG is a potential alternative for the replacement of yellow artificial dyes in gelled food products.

1. Introduction

Curcumin is a hydrophobic polyphenol obtained from the rhizomes of *Curcuma longa*, that presents a strong yellowish color and a wide range of beneficial biological activities such as anti-inflammatory, anticancer, anti-microbial and neuroprotective properties (Anand, Kunnumakkara, Newman, & Aggarwal, 2007). Due to these properties, and also to its low toxicity, curcumin is a valuable ingredient to be used by the food industry, as a natural yellow pigment to replace allergenic artificial dyes (Anand et al., 2007; Basnet & Skalko-Basnet, 2011). Despite these characteristics, the application of this compound has been limited due to its high hydrophobicity, poor absorption, low bioavailability and spicy flavor, which can affect the sensory properties of food products (Anand et al., 2007).

Among the techniques used to overcome these functional disadvantages, it is the encapsulation of curcumin in lipid carriers, such as the solid lipid particles (SLP). SLP are colloidal systems, similar to oil-in-water emulsions, in which the oil phase is replaced by solid lipids (La Torre & Pinho, 2015). Besides its capacity to encapsulate, protect and deliver lipophilic functional components, the SLP present many advantages including the possibility of promoting controlled release of bioactive compounds in the absence of organic solvents, and also the possibility of large scale production at a relatively low cost (La Torre & Pinho, 2015; Mehnert & Mäder, 2001).

Although the application of SLP may appear appealing to the food industry, special attention is required regarding the sensory characteristic of products containing SLP dispersions (Chojnicka-Paszun, Doussinault, & de Jongh, 2014). In products such as yogurts, dairy desserts, and starch puddings, for example, the incorporation of SLP dispersions may cause undesirable textural changes and decrease their acceptability. In such cases, the development of emulsion filled gels (EFG), which consist of dispersed lipid droplets/particles entrapped in a gelled matrix, could provide a viable alternative to overcome the textural problems (Liu, Stieger, van der Linden, & van de Velde, 2015;

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Oliver, Scholten, & van Aken, 2015; Sala, Wijk, van de Velde, & van Aken, 2008). These complex systems have been largely investigated, as they present rheological and breakdown properties strongly affected by the characteristics of the gel matrix, lipid fillers and the interactions among the components (Lorenzo, Zaritzky, & Califano, 2013; van Aken, Oliver, & Scholten, 2015).

Although a wide range of ingredients may be used to produce EFG, these systems are typically prepared using proteins and polysaccharides (Lorenzo et al., 2013; Vilela, Cavallieri, & Cunha, 2011). In addition, the combined application of these biopolymers may increase the gelling capacity of protein ingredients as the polysaccharides are, under some conditions, able to stabilize protein structures (Lorenzo et al., 2013; Vilela et al., 2011).

Among the protein ingredients extensively applied in food preparations is the soy protein isolate (SPI), which has a low cost, high nutritional value and good functional properties, such as the ability to form cold-set gels (Maltais, Remondetto, & Subirade, 2009). Cold-set gelation methods have been increasingly investigated because they allow the incorporation of thermal-sensitive compounds and promote the formation of gel structures in foods, without the need of heating the final product (Alting, de Jongh, Visschers, & Simons, 2002). Although some studies have applied cold-set methods to produce SPI gels (Maltais et al., 2009; Maltais, Remondetto, Gonzalez, & Subirade, 2005; Maltais, Remondetto, & Subirade, 2008), the addition of polysaccharides to these systems was not largely investigated (Chang, Li, Wang, Bi, & Adhikari, 2014; Vilela et al., 2011).

It is known that polysaccharides, such as xanthan gum (XG), may be used for the production of cold-set mixed gels through its incorporation to the protein solution before the second step of the gelation process (Chang et al., 2014; Jong, Jan Klok, & Van De Velde, 2009). XG is an anionic microbial polysaccharide, with high molecular weight (average molecular weight exceeds 10^6 Da), capable of forming mixed gels with protein ingredients, originating systems with different microstructural and rheological properties, which certainly has the potential for the development of new food products (Bertrand & Turgeon, 2007; Bryant & McClements, 2000; Chang et al., 2014).

In this context, the main objective of this study was to investigate the feasibility of encapsulating curcumin in solid lipid microparticles (SLM) incorporated in cold-set EFG, produced with SPI and XG. For this purpose, the capacity of SPI to form mixed gels with XG under cold-set conditions was evaluated and the study of the effect of SLM incorporation to the gels was carried out. In addition, the stability of curcumin encapsulated in SLM incorporated in the EFG was evaluated using instrumental colorimetry.

2. Material and methods

2.1. Chemicals and reagents

Soy protein isolate (SPI, Protimarti M-90, 84.3% protein) was obtained from Marsul (Montenegro, RS, Brazil), and Xanthan Gum (Grindsted Xanthan 80®) was donated by Danisco (Cotia, SP, Brazil). For the production of solid lipid microparticles, palm stearin (melting point = 50.1 °C) was donated by Agropalma (Belém, PA, Brazil), and Tween 80, Span 80 and curcumin (CAS 7727) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were reagent grade. Ultra-pure water (from a Direct Q3 system, Millipore, Billerica, MA, USA) was used throughout the experiments.

2.2. Production of curcumin-loaded solid lipid microparticles (SLM)

Curcumin-loaded SLM were produced using palm stearin as the lipid phase, and Tween 80 and Span 80 as hydrophilic and lipophilic surfactants, respectively. Initially, the lipid phase, consisting of palm stearin (4.5%, w/w) and span 80 (2.7%, w/w), was maintained at 80 °C for 30 min in order to eliminate the lipid thermal memory. Afterwards, curcumin (0.03%, w/w) was added to the lipid phase, and the aqueous phase, containing tween 80 (1.8%, w/w) dispersed in deionized water, was poured in the lipid phase. The resulting mixture was mixed using a rotor-stator device (T25, IKA, Staufen, Germany) at 18,000 rpm for 5 min and 80 °C. Sodium benzoate (0.02% m/m) was added to the samples to avoid microbiological spoilage. Immediately after preparation, the samples were subjected to centrifugation at 95*g* for 5 min at 25 °C (centrifuge Z-216 MK, Hermle, Wehingen, Germany) to remove non-encapsulated curcumin. All samples were prepared in triplicate and stored at a controlled temperature of 10 °C. The average particle size was obtained by a laser diffraction technique (SALD-201 V, Shimadzu, Kyoto, Japan).

2.3. Production of cold-set mixed gels

The production of cold-set mixed gels was performed according to protocol adapted from Maltais et al. (2008). The SPI was hydrated to obtain samples with concentrations 25% higher than the final concentrations desired. Next, the samples had the pH adjusted to 7, were preheated up to 80 °C for 30 min and cooled to room temperature. Then, a concentrated solution of the polysaccharide (0.6%, w/v) was added to the SPI dispersions, which were, subsequently, diluted in a concentrated $CaCl_2$ solution to the final desired concentration of protein, salt and polysaccharide. For the production of the concentrated solution of XG, the polysaccharide was hydrated with deionized water and subjected to magnetic stirring for 2 h, according to protocol adapted from Chang et al. (2014). In order to select the best formulation for the production of the mixed gels, different concentrations of SPI (5–15%, w/v), $CaCl_2$ (0–15 mM) and XG (0.1–0.3%, w/v) were tested and visual phase diagrams were constructed (Perrechil, Sato, & Cunha, 2011). The systems were classified according to their visual appearance as: low viscosity dispersions, viscous dispersions, high viscosity dispersions, non self-supported gels and self-supported gels. The formulation G_{XG} (15%, w/v SPI, 0.1%, w/v XG and 5 mM $CaCl_2$) was selected for the preparation of EFG. For this purpose, different volumetric percentages (50%, 75%, and 100%) of deionized water used to hydrate SPI were replaced by SLM dispersions. After preparation, the samples were stored at 10 °C for 15 h, and visual phase diagrams were constructed. The formulation G_{XG} was also used for the preparation of non-filled gels with free (non-encapsulated) curcumin and, for this purpose, the bioactive was added to the SPI dispersion before the preheating process.

2.4. Texture profile analyses (TPA)

TPA was performed using a texturometer (TA-XT.plus Texture Analyser, Godalming, Surrey, UK), with pretest speed of 3 mm/s, test and post-test speed of 1 mm/s, and 5 mm compression. Gels with 20 mm height and 20 mm diameter were compressed by an aluminum probe (20 mm diameter). Each formulation was analyzed in six replicates, and the parameters hardness, springiness, and cohesiveness were analyzed using the *Exponent* software incorporated in the equipment.

2.5. Water holding capacity (WHC)

WHC analyses were performed according to Beuschel, Culbertson, Partridge, and Smith (1992). For this purpose, the gel samples were weighed on Whatman paper number 1, placed in a falcon tube type and centrifuged at 6*g* for 10 min at 6 °C (Hermle centrifuge Labortechnik GmbH, model Z-216 MK, Wehingen, Germany). Subsequently, the samples were removed from the filters and the masses of the papers were determined. WHC values were then calculated according to the Eq. (1).