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Physicochemical stability of lycopene-loaded emulsions stabilized by plant or dairy proteins

Kacie K.H.Y. Ho[a,b], Karin Schroën[a], M. Fernanda San Martín-González[b], Claire C. Berton-Carabin[a,*]

[a] Food Process Engineering Group, Department of Agrotechnology and Food Sciences, Wageningen University, Bornse Weilanden 9, 6708 WG Wageningen, The Netherlands

[b] Department of Food Science, College of Agriculture, Purdue University, 745 Agriculture Mall Drive, West Lafayette, IN 47907, USA

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ABSTRACT

Lycopene is a lipophilic bioactive compound that can be challenging to deliver *in vivo*. To mediate this, delivery strategies, such as protein-stabilized oil-in-water (O/W) emulsions, have been suggested to improve the physicochemical stability and bioavailability of lycopene. In this research, the effects of plant (soy and pea) and dairy (whey and sodium caseinate) proteins on physical stability (droplet size, charge, interfacial rheology) and lycopene retention in canola O/W emulsions (pH = 7.0, 10% oil) were compared.

Particle size distribution for sodium caseinate and pea protein-stabilized emulsions remained unchanged after 14 days of refrigerated storage, while whey and soy protein isolate-stabilized emulsions became unstable. Zeta potential was largely negative (−45 to −60 mV) for all emulsions and the lycopene concentration in plant protein-stabilized emulsions at 14 days of storage was similar to that in sodium caseinate-stabilized emulsions. Sodium caseinate formed relatively viscous films at the oil-water interface, while the other proteins formed more elastic layers. Despite this difference, both the caseinate and pea protein-stabilized emulsions were promising delivery vehicles, indicating that plant-derived proteins can be feasible alternatives to dairy emulsifiers.

1. Introduction

Recently, there has been growing interest in enriching food products with bioactive ingredients (e.g., flavors, vitamins, antioxidants or phytochemicals) to produce a desired functionality. Lycopene is the most potent singlet oxygen quencher amongst carotenoids (Di Mascio, Kaiser, & Sies, 1989; Rao, Waseem, & Agarwal, 1998) that could be used as a naturally derived antioxidant or as a health-promoting ingredient. However, lycopene is largely insoluble in water and chemically labile. Therefore, encapsulation strategies should be considered, such as using emulsion-based delivery systems.

Dairy proteins have been extensively used for food applications, and in particular to stabilize the interface in oil-in-water (O/W) emulsions. Compared to other emulsifiers (e.g., surfactants or modified starch), dairy proteins, such as whey protein isolate (WPI) and sodium caseinate (SC), can improve the physical and chemical stability of carotenoid-loaded emulsions (Mao et al., 2009; Mao, Yang, Yuan, & Gao, 2010). The high colloidal stability is attributed to the ability of dairy proteins to form thick and sterically-stabilized interfacial layers (Dickinson, 2001). In emulsion stability, the interfacial protein layer plays a critical role in the physical stabilization process (Wilde, 2000). Amongst dairy proteins, whey proteins (mostly represented by the globular protein β-lactoglobulin) have a rigid structure, which is known to lead to different interfacial organization compared to SC (primarily β-casein), which has a flexible structure (Dickinson, 2013) and in turn may lead to different effects on the physical and perhaps chemical stability of emulsions. Besides, Cornacchia and Roos (2011) found that the different protein chemistries of WPI and SC affected β-carotene retention in O/W emulsions, with the latter protein providing a better oxidative barrier. Dairy protein emulsifiers have also proved to promote the bioavailability of bioactives. Ribeiro et al. (2006) found that interfacial WPI combined with Tween 20 or sucrose laurate improved cellular uptake of lycopene and astaxanthin, compared to emulsions stabilized with only Tween 20 in colon carcinoma cells (lines HT-29 and Caco-2). Although the

* Corresponding author.
E-mail addresses: ho47@purdue.edu (K.K.H.Y. Ho), karin.schroen@wur.nl (K. Schroën), msanmart@purdue.edu (M. F. San Martín-González), claire.carabin-berton@wur.nl (C.C. Berton-Carabin).

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mechanism of enhanced bioavailability was not elucidated, the authors alluded to potential interactions between the carotenoids and β-lactoglobulin as a possible explanation.

The drawback of using dairy proteins for producing functional food emulsions is their low sustainability and impact on the environment (Erb et al., 2016; VandeHaar & St-Pierre, 2006). Plant proteins represent a large and relatively underutilized resource that is more sustainable and requires less energy for production compared to their animal-derived counterpart (de Boer, Helms, & Aiking, 2006; O'Kane, Vereijken, Happe, Gruppen, & J S Van Boekel, 2004). Recent reviews (Shi & Dumont, 2014; Song, Tang, Wang, & Wang, 2011) have also highlighted functional properties of different biobased films from plant proteins as the utilization of such renewable proteins has gained popularity. Despite the growing interest for plant-derived proteins as emulsifiers (Chihi, Mession, Sok, & Saurel, 2016), the link with stabilization of bioactive components in O/W delivery systems is hardly ever made. Many plant proteins, including soy protein isolate (SPI) and pea protein isolate (PPI) have been reported as promising functional emulsifiers (Aoki, Taneyama, & Inami, 1980; Bengoechea, Cordobés, & Guerrero, 2006; Lam & Nickerson, 2013; Pelgrom, Berghout, Van Der Goot, Boom, & Schutyser, 2014; Phoon, San Martin-Gonzalez, & Narsimhan, 2014), yet it is still arguable whether they perform as well as dairy proteins, or even outperform them (Chove, Grandison, & Lewis, 2001). SPI and PPI are both from commonly consumed plant sources and exhibit good emulsifying properties as they have been shown to form stable O/W droplets that were not drastically bigger compared to β-lactoglobulin-stabilized droplets (Benjamin, Silcock, Beauchamp, Buettner, & Everett, 2014). Interfacial properties of SPI and PPI have also been studied and demonstrate potential to physically stabilize O/W emulsions by forming strong viscoelastic films (Chang et al., 2015). Despite the numerous studies characterizing soy and pea protein functionality, limited work (Fernandez-Avila, Arranz, Guri, Trujillo, & Corredig, 2016; Tapal & Tiku, 2012) has been conducted specifically on SPI, consisting primarily of globular proteins glycinin and conglycinin (Chronakis, 1996), and PPI, consisting primarily of legumin and vicilin/convicilin (O'Kane et al., 2004), for improving bioactive delivery. Tapal and Tiku (2012) conducted research on curcumin and SPI complexation and found that >80% of the bioactive was retained during simulated gastric conditions. Fernandez-Avila et al. (2016) also found promising results for plant protein (SPI and PPI)-stabilized emulsions, as conjugated linoleic acid (CLA) delivery was enhanced compared against non-emulsified CLA for both proteins in a Caco-2 cell model. Despite these promising first results, it is still unknown whether plant proteins could be a valuable alternative to dairy proteins for the production of functional emulsions loaded with bioactives, such as lycopene. In fact, direct comparisons between plant and dairy proteins and the link between interfacial properties and bioactive encapsulation have hardly been touched upon.

For the design of emulsion-based encapsulation systems, we believe it is necessary to connect the physicochemical stability of emulsions with the structural organization of the oil-water interface. Consequently, the aims of this study were to determine the effect of interfacial dairy or plant protein on the: 1) physical stability (particle size and zeta potential) and 2) chemical stability (lycopene retention) of emulsions, and 3) interfacial organization (adsorption kinetics and dilatational rheology). Ultimately, we have attempted to relate these findings and provide guidelines for the design of sustainable protein-stabilized emulsion-based delivery systems.

2. Materials and methods

2.1. Materials

Canola oil and tomato paste for lycopene extraction were purchased from local supermarkets (Wageningen, Netherlands). MP Alumina N-Super I (MP Biomedicals, France) was mixed with canola oil overnight as previously described (Berton, Genot, & Ropers, 2011) to strip the oil of tocopherols and surface-active impurities. All-*trans*-lycopene standard, all solvents (analytical grade) and other reagents were purchased from Sigma Aldrich (Zwijndrecht, the Netherlands). Proteins were generously donated by the suppliers as follows: 97.5% purity WPI (BIPRO, Davisco, Switzerland), 80% purity SC (Sodium Caseinate S, DMV International, Amersfoort, Netherlands), and 90% purity SPI (soy protein isolate SUPRO EX 37, Solae Europe SA, Switzerland) and 80–90% PPI (pea protein isolate NUTRALYS F85, Roquette, France). Ultrapure water (Millipore Milli-Q water purification system) was used for all experiments.

2.2. Methods

2.2.1. Preparation of lycopene oil stock

Approximately 250 g of tomato paste were combined with 10 g of celite, 10 g of sodium bicarbonate, and 500 mL of an extraction solvent (1:1 v/v hexane (0.1% butylated hydroxytoluene w/v) – ethyl acetate). The mixture was held under a stream of nitrogen and in an ice-bath while stirring at 250 rpm with an overhead IKA mixer for 1.5 hours. The mixture was then vacuum filtered with No. 1 filter paper (Whatman, United Kingdom) to separate solids from liquids, transferred to a separatory funnel, and washed with a saturated solution of sodium chloride in water. The lower aqueous phase was drained and the upper hexane layer was collected, flushed with nitrogen and rotary evaporated almost to dryness. Stripped oil (~80 g) was added to solubilize the lycopene crystals prior to transferring to a borosilicate screw top bottle. The resulting lycopene-in-oil mixture was held under a stream of nitrogen to remove residual solvent until constant weight was achieved. This entire process was repeated 10 times and individual batches of lycopene oil were pooled, prior to aliquoting into 35 mL batches, flushing with nitrogen, and storing at −20° C. The lycopene content of the stock oil was determined after dilution in hexane spectrophotometrically at 471 nm, analyzed using high-performance liquid chromatography (Kean, Hamaker, & Ferruzzi, 2008), and then compared against an *all*-trans-lycopene standard to identify *cis*- and *trans*- isomers (Ho, Ferruzzi, Liceaga, & San Martín-González, 2015). The resulting stock oil had a total lycopene concentration of 0.236 mg/g of oil and consisted primarily of all-*trans*-lycopene (~90%).

2.2.2. Preparation of the aqueous phase

WPI and SC were added to 0.01 M phosphate buffer (pH = 7) and stirred with a magnetic stir bar overnight at room temperature at 100 rpm prior to emulsification the following morning. Preliminary experiments showed SPI and PPI contained an insoluble fraction (Supplementary data, Table A.1), which negatively affected the emulsifying properties. Hence, the nonsoluble fraction of the plant protein was removed prior to emulsification. SPI or PPI were combined with 0.01 M phosphate buffer (pH = 7) and stirred for 48 h at 200 rpm at 4° C. The resulting mixtures were centrifuged at 10,000 × *g* for 10 min at 20° C. The supernatant was collected and centrifuged again under the same conditions. The resulting supernatant, containing the soluble protein fraction, was carefully collected and stored at 4° C prior to use. The soluble protein concentration was estimated following a standard protocol for BCA Protein Assay (Thermoscientific, 2015). The removed pellet,