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Physical properties and release behaviour of caffeine multiple emulsions stabilised by binary or ternary biopolymer soluble complexes under acid, bile and yogurt storage conditions

Nancy Y. Hernández-Marín [a], Consuelo Lobato-Calleros [b, *], Angélica Román-Guerrero [c], Jose Alvarez-Ramirez [d], E. Jaime Vernon-Carter [d]

[a] Posgrado en Ciencia y Tecnología Agroalimentaria, Universidad Autónoma Chapingo, Km. 38.5 Carretera México-Texcoco, 56230 Texcoco, Mexico
[b] Departamento de Preparatoria Agrícola, Universidad Autónoma Chapingo, Km. 38.5 Carretera México-Texcoco, 56230 Texcoco, Mexico
[c] Departamento de Biotecnología, Universidad Autónoma Metropolitana-Iztapalapa, San Rafael Atlixco 186, Vicentina, México, D.F. 09340, Mexico
[d] Departamento de Ingeniería de Procesos e Hidráulica, Universidad Autónoma Metropolitana-Iztapalapa, San Rafael Atlixco 186, Vicentina, México, D.F. 09340, Mexico

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ABSTRACT

Whey protein (W) and/or whey peptides (P) and carboxymethylcellulose (C) soluble complexes were designed and used for stabilising water-in-oil-in-water multiple emulsions (ME's) loaded with caffeine in the inner aqueous phase. Interfacial layer composition of the ME's affected the initial droplet size, bulk viscoelastic properties, and caffeine release rates in yogurt and under acid and bile conditions. Oil droplet size of all the ME's did not vary significantly over 21 days of storage. The molecular weight of the protein fraction predominating in the protein:polysaccharide soluble complex was a key factor in determining the ME's properties. When W predominated in the soluble complexes, as opposed to P, initial droplet size was smaller, the viscoelastic properties higher, and the caffeine release slower in yogurt and under acid and bile conditions.

1. Introduction

Caffeine is a naturally occurring alkaloid compound and the most frequently consumed central nervous system stimulant in the world, and has been targeted for its inclusion in a growing number of foods and beverages by food manufacturers (Fulgoni, Keast, & Lieberman, 2015). However, caffeine inclusion in foods presents two drawbacks: (i) its pronounced bitterness and astringency (Belščak-Cvitanović et al., 2015); and (ii) its rapid absorption into the blood stream and immediate effect on the central nervous system (Martínez-López, Sarriá, Baeza, Mateos, & Bravo-Clemente, 2014). Thus, an ongoing research topic is to design suitable caffeine encapsulation systems that may help to overcome these problems (Matoušková, Patočková, Doskočil, & Márová, 2012). Water-in-oil-in-water multiple emulsions (ME's) are multiple compartment structure systems where bioactive components could be encapsulated within the inner water phase, the intermediate oil phase, or the outer water phase (Leal-Calderon, Schmitt, & Bibette, 2007). In particular, the presence of serial interfaces to be crossed by a hydrophilic encapsulated compound in the internal aqueous phase of the ME before reaching the external phase is assumed to slow down the release process (Doucet, Ferrero, Garcia, & Zastrow, 1998). In practice, only the two-stage emulsification method can produce stable ME's of well-defined composition and reproducible droplet-size distribution (Dickinson & McClements, 1996). The use of a mixture of oil and water soluble emulsifiers with an appropriate hydrophilic-lipophilic balance permits the obtention of smaller and more stable inner water droplets (Garti, 1999; Tadros, 2013), while an improved stability and homogeneity in multiple droplet size distribution can be obtained by using protein:polysaccharide complexes at the external oil-water interface (Jiménez-Alvarado, Beristain, Medina-Torres, Román-Guerrero, & Vernon-Carter, 2009). Pre-formed protein-polysaccharide complexes for stabilizing O/W and ME's has gained considerable attention lately (Zinoviadou, Scholten, Moschakis, & Biliaderis, 2012), as protein-stabilized emulsions are usually prone to instabilities by pH, ionic

* Corresponding author.
E-mail address: consuelobato@yahoo.com (C. Lobato-Calleros).

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strength and temperature and their assembly with polysaccharides can strongly improve emulsion stability (Salminen & Weiss, 2014). The emulsifying properties of whey proteins have been enhanced by their complexation with polysaccharides (Li et al., 2012). In particular whey protein (W):carboxymethylcellulose (C) complexes have been found to increase the stability of O/W and ME's (Dickinson, 2008). Hernández-Marín, Lobato-Calleros, and Vernon-Carter (2013) determined the conditions leading to pre-formed electrostatic soluble complexes between W and C and demonstrated their ability to stabilise ME's. On the other hand, there is a trend in the food industry to incorporate bioactive whey peptides (P) into foods with the aim of promoting the health by means of a tailored diet (Nagpal et al., 2011). Thus, the particular interest in this work was to explore if the complexation between P, C and P, C, W could equal or enhance the emulsifying performance of W:C complexes. When formulating bioactives encapsulation systems, their stability into food products should be taken into consideration in order to evaluate their potential application in the development of functional foods. To our knowledge there are no reports about the release behaviour of bioactives from multiple emulsions when they are incorporated into more complex food products. Finally, it has been reported that the use of ME's allows to reduce significantly the fat content of dairy products based on a milk-protein gel structure, like yogurt (Lobato-Calleros, Rodríguez, Sandoval-Castilla, Vernon-Carter, & Alvarez-Ramirez, 2006).

With these premises in mind, the objectives of this work were to: (1) stabilise and evaluate the physical properties of caffeine loaded multiple emulsions stabilised by binary or ternary complexes between whey protein, carboxymethylcellulose and/or whey peptides; (2) to formulate reduced milk-fat yogurts variations incorporating the multiple emulsions in partial substitution of milk-fat; and (3) evaluate the caffeine release under acid, bile and yogurt storage conditions.

2. Materials and methods

2.1. Materials

Canola oil (CO; Maravilla, Aceites, Grasas y Derivados, S.A. de C.V., Mexico City, Mexico) was used as the oil phase of the ME. Grinsted® PGPR 90 (G), esters of polyglycerol and polyriciniolate fatty acids (PGPR; E-476; Danisco Mexico, S.A. de C.V., Mexico City, Mexico), and soy lecithin (L; Alquimia Mexicana, S. de R. L. Mexico City, Mexico) were used as emulsifiers to prepare the primary emulsion (W_1/O). Caffeine was purchased from Sigma-Aldrich Quimica, S.A. de C.V. (Toluca, State of Mexico, Mexico). Whey protein concentrate (W; isoelectric point (pI) of 4.2 ± 0.1; 83.7 wt % protein and 6.4 wt % fat, Hilmar 8000®, Hilmar, CA, USA), carboxymethylcellulose (C; CMC® CEROL; viscosity of 50 000 mPa s for a 2 wt % solution at 60 rpm and 25 °C; Grupo Dermet, S.A. de C.V., Mexico City, Mexico), and whey peptides (P; isoelectric point (pI) of 4.2 ± 0.1; 80.5 wt % protein, 5.8 wt % fat, 17 wt % peptides larger than 20 kg mol^{-1}, 15.6 wt% peptides falling between 5 and 20 kg mol^{-1}, and 67.2 wt % peptides smaller than 5 kg mol^{-1}; Hilmar 8360®, Hilmar, CA, USA) were used to prepare the soluble complexes, which were used to stabilise the ME's.

Low-heat skim milk (0.01 wt% fat, Lactomix®, DILAC, SA de CV, Mexico City, Mexico) and homogenised whole milk (28 wt% fat, Reny Picot, DILAC, SA de CV, Mexico City, Mexico) spray-dried powders, and freeze-dried starter culture (*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *lactis*, and *Lactobacillus delbrueckii* subsp. *bulgaricus* MY800 LYO Danisco, S. A. de C. V. Mexico City, Mexico) were used to prepare the stirred yogurts.

Reagents used were porcine bile extract (B8631, Sigma-Aldrich Co., St. Louis, MO, USA), hydrochloric acid (HCl) (J. T. Baker, SA de CV, Xalostoc, State of Mexico, Mexico). All water used in the experiments was double distilled and deionised.

2.2. Soluble complexes formation

In a previous work it was established that the best W:C weight ratio ($R_{W:C}$) for obtaining soluble complexes was 3:1 at a pH value of 4.25 (Hernández-Marín et al., 2013). It was also established that a concentration of 0.5 wt % of C for the soluble complexes formation was required for obtaining stable multiple emulsions. This is in agreement with findings of Malinauskyte and Leskauskaite (2013) regarding the use of whey proteins-carboxymethylcellulose complexes for stabilizing O/W emulsions, although the C used by these authors had a lower viscosity (1000–3000 mPa s at 2%, 25 °C). In this work, we determined the best P:C weight ratio ($R_{P:C}$) and pH leading to the formation of P:C soluble complexes. Briefly, $R_{P:C}$ ratios ranging from 6:1 to 10:1 were obtained by blending P (3.0, 3.5, 4.0, 4.5, and 5.0 wt %) solutions with a C (0.5 wt %) solution. A 0.1 N HCl solution was used to adjust the pH of the mixed solutions in the range of 2.0–6.0. The turbidity of each mixture left to stand for 48 h at 4 ± 1 °C was measured with a Spectronics Genesys 5 UV/Vis (Spectronic Unicam, Rochester, NY, USA) at a wavelength of 400.5 nm. Based on the best weight ratios and pH values leading to the soluble binary biopolymers complexes (W:C and P:C) formation, the conditions for ternary W:C:P biopolymer soluble complex formation were established, maintaining the concentration of C at 0.5 wt %.

2.3. Formulation and preparation of the ME's

ME's were prepared at room temperature (20 ± 2 °C) using a two-stage emulsification procedure (Lobato-Calleros et al., 2006). In the first stage, the primary (W_1/O) emulsion was made with a disperse mass fraction of 0.4. One hundred grams of W_1/O, was made up by an aqueous phase (39.24 g of distilled water + 0.52 g of L + 0.24 g of caffeine) that was added drop-wise to the oil phase (56.52 g of CO + 3.48 g of G) using a high shear Ultra-Turrax® T50 basic homogeniser (IKA Works, Inc., Wilmington, DE, USA) operated at 6400 rpm during 5 min. In the second stage a mass fraction of 0.3 of W_1/O emulsion was re-emulsified in pre-formed binary (W:C or P:C) or ternary (W:C:P) biopolymers soluble complexes aqueous solutions (W_2), which were obtained using the biopolymers concentrations and pH established from the experiments of subsection 2.2. Homogenisation was done with the high shear homogeniser operated at 4000 rpm during 5 min. The resulting multiple emulsions were coded as $ME_{P:C}$, $ME_{W:C}$, and $ME_{W:C:P}$. The ME's were prepared by triplicate using a completely randomised design, and stored at 4 °C until required for analyses.

2.4. Mean droplet size and optical micrographs of the emulsions

The mean hydrodynamic diametre (d_h) of the primary emulsion was determined immediately after formation and after 21 d of storage at 4 °C using a Malvern Zetasizer Nano ZS90 (Malvern Instruments Ltd., Malvern, Worcestershire, UK). The mean surface-volume diametre ($d_{3,2}$) and particle size distribution (PSD) of the ME's were determined with a Malvern Mastersizer 3000 (Malvern Instruments Ltd., Malvern, Worcestershire, UK), using deionised water (refractive index 1.333) as dispersant within 1 h of initial preparation and at different intervals during 21 days of storage. Micrographs of the ME's were taken after 1 h of preparation and after 21 days of storage using an optical microscope (Olympus BX45, Olympus Optical Co., Tokyo, Japan) coupled to an image analyser system (digital Olympus camera C3030, Olympus Imaging Americas Inc., Center Valley, PA, USA). Selected micrographs taken