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Rheological and release properties of double nano-emulsions containing crocin prepared with Angum gum, Arabic gum and whey protein

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ABSTRACT

Crocin (a bioactive of saffron) is a highly water soluble carotenoid with several physiological benefits. It is sensitive to environmental conditions such as light, oxygen and pH. In this study, firstly a W_1/O microemulsion containing crocin in W_1 phase was prepared using spontaneous method and then, double emulsions ($W_1/O/W_2$) prepared with Angum gum (AG) in outer aqueous phase (W_2) and compared with whey protein concentrate (WPC) and gum Arabic (GA). Emulsions containing Angum gum showed highest viscosity (about 10 times higher) and gel like behavior comparing to GA. Droplet size of W_1/O microemulsions was approximately 10 nm on average. WPC and GA produced double emulsions with droplet diameter of 429 and 695 nm, respectively for 5% biopolymers. Although the highest droplet size was seen in Angum gum stabilized emulsions, they showed the lowest creaming and highest stability which could be attributed to its high viscosity. In terms of dynamic behavior, for all samples storage modulus (G′) was higher than loss modulus (G″).

1. Introduction

Crocins are a group of hydrophilic carotenoids, constitute coloring part of Saffron (Jafari, Mahdavi-Khazaei, & Hemmati-Kakhki, 2016; Rajabi, Ghorbani, Jafari, Sadeghi Mahoonak, & Rajabzadeh, 2015; Sarfarazi, Jafari, & Rajabzadeh, 2015). The crocin families are glycosyl esters of crocetin (a dicarboxylic acid) which six types of them have been detected in saffron. Among different crocins, all-trans crocetin di-β-D-gentiobiosyl ester or crocin posses highest coloring capacity due to its high water solubility (Alonso, Salinas, Garijo, & SÁNchez-FernÁNdez, 2001; Melnyk, Wang, & Marcone, 2010; Srivastava, Ahmed, Dixit, Dharamveer, & Saraf, 2010). Crocin has high antioxidant capacity and proven beneficial effects on many organs including nervous system, gastrointestinal, cardiovascular, genial, endocrine and immune systems (Hosseinzadeh & Ghenaati, 2006; Bandegi, Vafaei Abbas, Ghaderdoost, & Rashidy-Pour, 2011; Akhtari et al., 2013; Karami et al., 2013; Alavizadeh & Hosseinzadeh, 2014; Ghaeni, Amin, Hariri, Meybodi, & Hosseinzadeh, 2014; Shahi, Assadpour, & Jafari, 2016). Crocin has low stability and after exposure to heat, oxygen, light, acidic environment and presence of additives during processing and storage of foods, most of its functionality is lost (Rahaiee, Shojaosadati, Hashemi, Moini, & Razavi, 2015).

Multiple emulsions are a group of structured emulsions, consist of small droplets of one phase embedded within larger droplets of another phase that are themselves dispersed in a continuous phase like water-in-oil-in-water ($W_1/O/W_2$) and oil-in-water-in-oil ($O_1/W/O_2$) (Assadpour, Maghsoudlou, Jafari, Ghorbani, & Aalami, 2016a, b). $W_1/O/W_2$ emulsions are made up of a water in oil primary emulsion (W_1/O) that itself is dispersed in another aqueous phase (W_2). $W_1/O/W_2$ emulsions have applications in formulation of reduced-fat food products and as vehicles for encapsulation and delivery of hydrophilic bioactive compounds and drugs (Dickinson, 2011; McClements, 2015a, 2015b). Increasing stability and controlling release of active materials and drugs in aqueous inner phase (W_1) are the main challenges of producing double emulsions (Benichou, Aserin, & Garti, 2004, 2007; Hemar, Cheng, Oliver,

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Sanguansri, & Augustin, 2010; Lutz, Aserin, Wicker, & Garti, 2009; Murillo-Martínez, Pedroza-Islas, Lobato-Calleros, Martínez-Ferez, & Vernon-Carter, 2011).

Angum gum which also is known as Persian gum or Zedu is exudates of the wild or mountain almond tree (*Amygdalus scoparia* Spach). The main Angum gum producer is Iran with over 400 t of annual export which mostly is used in food, medicinal and industrial applications. Previous studies showed lower emulsifying ability and higher emulsion stability of Angum gum comparing with Arabic gum that is mostly due to higher viscosity of Angum gum stabilized emulsions (Abbasi & Mohammadi, 2013; Jafari, Beheshti, & Assadpoor, 2012, 2013). Angum gum is an acidic gum (pH 4.4 to 5.6) with protein content of 0.2–0.6%, carbohydrate content of 87%, about 8% moisture and 2.5 ash (Golkar, Nasirpour, & Keramat, 2015). Backbone of Angum gum is composed of galactose (1 → 3 linked β-D-Galp) and rhamnose whereas the branches are composed of (1 → 6) linked β-D-Galp and (1→) and/or (1 → 3) linked α-L-Araf residues (Mohammadi, Abbasi, & Scanlon, 2016). Gum Arabic (GA) is the most commonly used biopolymer emulsifier in the flavor beverage emulsions. It is derived from the natural bark exudate of *Acacia senegal* and consists of at least 3 high molecular weight biopolymer fraction (Hosseini, Jafari, Mirzaei, Asghari, & Akhavan, 2015). The surface active fraction is believed to consist of branched arabinogalactan blocks attached to a hydrophobe polypeptide backbone. The hydrophobic part anchors the molecule to the droplet surface and hydrophilic part extends into the solution and provides stability against droplet aggregation through steric and electrostatic repulsion (Ozturk, Argin, Ozilgen, & McClements, 2015). The average molecular weight of GA is considered to be about 3–5.8 × 10^5 (300–800 kDa). It contains three parts, i.e. arabinogalactan (80–90%), glycoprotein (2–4%) and arabniogalactan-protein (10–20) (Idris, Williams, & Phillips, 1998; Klein, Aserin, Ishai, & Garti, 2010). It facilitates formation of small droplets by lowering interfacial tension during homogenization (Buffo, Reineccius, & Oehlert, 2001). Protein component comprise 2 wt% of GA molecule (McNamee, O'Riorda, & O'Sullivan, 1998).

Whey protein concentrate (WPC) is a mixture of different globular proteins with β-lactoglobulin with molecular weight of 18.3 × 10^3 g/mol being the most dominant followed by α-lactalbumin (Ozturk et al., 2015). The ability of whey protein to form stable emulsions depends on emulsion composition (including pH, mineral content, salt, sugar, surfactant and polysaccharide content) and environmental conditions (homogenization temperature and pressure) (Assadpour, Jafari, & Maghsoudlou, 2017; Assadpour & Jafari, 2017).

The aim of this study was preparing crocin loaded double emulsions using Angum gum and comparing its rheological and release properties with WPC and GA prepared double emulsion.

2. Materials and methods

Crocin was purchased from Sigma-Aldrich Co. (St. Louis, MO), polyglycerol polyrecinoleate (PGPR) 4175 kindly donated by Palsgaard. Arabic gum were purchased from Samchun Chemical Co. (South Korea). WPC was purchased from Arla Food Ingredient (Viby, J, Denmark) and based on Arla Food datasheet contains 76–80% protein and Maximum 9% lactose. Extra virgin olive oil purchased from local market. Angum gum was purchased from a herbal store (Shiraz, Iran).

2.1. Preparation of W_1/O emulsions

Water in oil primary microemulsions was prepared using spontaneous method for production of micro-emulsions. Briefly, 10 wt% PGPR (HLB = 1.5) was mixed with 80 wt% olive oil using magnetic stirrer (1000 rpm) for 15 min at ambient temperature (25 °C). Then 10 wt% water containing 0.2% crocin was added to the mixture of PGPR and olive oil in 1.5 h while stirring at 700 rpm (Mehrnia, Jafari, Makhmal-Zadeh, & Maghsoudlou, 2016).

2.2. Extraction, purification and drying of Angum gum

White to amber color particulates of gum were separated and their bark residues and debris removed using a sharp knife. Angum gum was prepared according to method of Jafari et al. (2013). Briefly a batch of Angum gum was selected and powdered using a lab mill (IKA, A11 basic). Powdered sample was extracted using 96% v/v ethanol for 6 h in a Soxhlet apparatus. Then it was dried for 12 h at 40 °C and rehydrated with distilled water and heated to 50–60 °C with simultaneous stirring. Final solution was filtered through Whatman filter paper No 2 using a vacuum pump to remove insoluble parts of gum. Filtrate was dried at 40 °C.

2.3. Preparation of $W_1/O/W_2$ emulsions

Double emulsions were prepared using two stage emulsification of W_1/O microemulsion (10 wt%) in outer aqueous phase containing biopolymers (W_2). At first, outer aqueous phase prepared by dissolving WPC and GA (final concentration of 5 and 10 wt%) and AG (final concentration of 2.5 and 5 wt%, due to its high viscosity and better homogenization process) in distilled water containing 0.01 wt% sodium azide and using HCl and NaOH, their pH was adjusted on 7. Coarse $W_1/O/W_2$ emulsion prepared with an Ultra Turrax homogenizer (IKA, T25, Germany) at 8000 rpm for 10 min. Final emulsion prepared using high pressure valve homogenizer (Emulsiflex-C3, Avstin, CA) at 10000 psi for 3 cycles and ambient temperature.

2.4. Droplet size analysis and microstructure

Droplet size of W_1/O and $W_1/O/W_2$ emulsions was measured using a dynamic light scattering method (Zetasizer Nano Zs, Malvern Instrument, Malvern, UK). To avoid multiple scattering, W_1/O emulsions and $W_1/O/W_2$ emulsions were diluted using olive oil (detection angle = 173°, refractive index = 1.463, viscosity = 0.08 Pa s and density = 910 kg/m^3) and distilled water (1:1000), respectively. Diluted double emulsions were observed using Olympus CX21 microscope (Tokyo, Japan) with 40× objective lens, equipped with a video camera. All measurements were conducted after overnight storage of samples at ambient temperature.

2.5. Emulsion stability

The stability of emulsions was evaluated by pouring double emulsions into glass tubes (1 cm diameter and 15 cm height) to the height of 10 cm and tightly sealed with plastic caps and stored at ambient temperature. The emulsions stability index (ESI) was defined as a relative ratio of Z-average for stored emulsion to the fresh emulsions.

2.6. Release properties

Crocin release from different double emulsions was studied in gastric (pH 1.2, HCl 0.1 M) and intestine (pH 6.8, KH_2PO_4) solution using dialysis bag method at 37 °C and 100 rpm without any digestive enzymes. 3 ml of double emulsions was poured into dialysis bag (D0655, Sigma, Canada) with 12.4 kDa cut-off. The bags then placed into 50 ml gastric buffer for 2 h and subsequently were placed in 60 ml intestinal buffer for 6 h. At certain time intervals (6 times in first 2 h and 8 times in intestinal buffer), released crocin