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Ultrasound-assisted formation of the canthaxanthin emulsions stabilized by arabic and xanthan gums

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ABSTRACT

There is interest in incorporating canthaxanthin (CTX) into food emulsions due to its high potential health benefits. The used CTX in this study was produced by the bacterium of *Dietzia natronolimnaea* HS-1. Then, the influence of main emulsion components (gum arabic (GA), xanthan gum (XG) and coconut oil (CO)) on the surface-weighted mean diameter (D_{32}), polydispersity index (PDI), specific surface area (SSA) of droplets and density of the emulsions containing CTX was optimized using response surface methodology (RSM). Polynomial equations between the responses and independent variables were derived. The linear effect of GA had a significant ($p < 0.0001$) term in all reduced models. The optimal formulation for emulsions was composed of GA content of 9.85% (w/w), XG content of 0.13% (w/w) and CO concentration of 3.50% (w/w). This optimum formulation yielded D_{32} of 0.752 μm, PDI of 1.533, SSA of 9.995 m^2/ml and density of 1.0357 g/cm^3.

1. Introduction

Oil-in-water (O/W) emulsions are considered to be very important fluids due to their specific properties and applications in medicine and the petroleum, chemical, and food industries (Huang, Kakuda, & Cui, 2001; Rousseau, 2000). Since the contact between oil and water molecules is energetically unfavorable, emulsions are thermodynamically unstable systems (Harnsilawat, Pongsawatmanit, & Mcclements, 2006). Emulsion stability is characterized in various mechanisms – creaming or sedimentation, flocculation of droplets, coalescence between droplets, or phase separation (Gharibzahedi, Mousavi, Hamedi, & Ghasemlou, 2012; Granato, Castro, Neves, & Masson, 2010). Stoke's law explains that the velocity at which a droplet moves is inversely proportional to the viscosity of water phase and directly proportional to the square of the droplet size radius and density difference between water and oil phases (Taherian, Fustier, & Ramaswamy, 2006). Therefore, reducing droplet size along with increasing density of oil droplet and viscosity of water phase can enhance the emulsion stability (Chanamai & McClements, 2000).

Hydrocolloids are used as stabilizers and emulsifiers in O/W emulsions. Most hydrocolloids can act as stabilizers (stabilizing agents) of the emulsions, but only a few can act as emulsifiers (emulsifying agents) (Harnsilawat et al., 2006). A suitable hydrocolloid for the concentrated emulsions need to have high emulsifying capacity, high solubility in cold water, low viscosity in solution, and no gelling and/or thickening effects for a special period of time (Dickinson, Galazka, & Anderson, 1991). It is well documented that gum arabic (GA), a natural polysaccharide, has excellent emulsification properties for some emulsions (Buffo, Reineccius, & Oehlert, 2001; Dłuzewska, Stobiecka, & Maszewska, 2006; Gharibzahedi, Mousavi, Hamedi, & Ghasemlou, 2012; Gharibzahedi, Mousavi, Hamedi, Khodaiyan, Razavi, 2012; Gharibzahedi, Mousavi, Khodaiyan, & Hamedi, 2012; Tan, 1990). This hydrocolloid is surface active, adsorbs to interfaces between oil and water, and facilitates the production of small droplets by lowering the interfacial tension during homogenization (Buffo et al., 2001). Xanthan gum (XG) is an anionic heteropolysaccharide produced by the bacterium, *Xanthomonas campestris* and has a particularly complicated molecular structure (Higiro, Herald, & Alavi, 2006). It has been extensively applied due to its excellent viscosity and dispersion (e.g. the reversible shear-thinning, dispersed in either hot or cold water) characteristics. Therefore, this biopolymer tends to form in solution structures, showing high low-shear and weak gel properties that lend stability to colloidal suspensions (Nikiforidis & Kiosseoglou, 2010). Gharibzahedi, Mousavi, Hamedi, Khodaiyan, et al. (2012) and Gharibzahedi, Mousavi, Hamedi, & Khodaiyan (2013) showed that the use of GA biopolymer in combination with XG can increase physicochemical stability of walnut oil-in-water emulsions.

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Functional foods are made up of natural components that impart nutritional benefits to human health. Carotenoids are examples of these components which, in addition to their inherent nutritional value, also contribute to the taste and colour of foods (Batista, Raymundo, Sousa, & Empis, 2006). Among the produced carotenoids, β-carotene and the xanthophylls astaxanthin, canthaxanthin (CTX), and lutein are the major compounds with particular commercial interest (Hojjati, Razavi, Rezaei, & Gilani, 2011). CTX (4,4′-diketo-β-carotene) is an orange-red ketocarotenoid with high antioxidant activity. Production of this pigment from biological resources with regarding to restriction of synthetic carotenoids in different industries has recently developed throughout in the world (Gharibzahedi, Razavi, & Mousavi, 2012; Gharibzahedi, Razavi, & Mousavi, Moayedi, 2012). Among the introduced sources, the bacterium of *Dietzia natronolimnaea* HS-1 is recognized as a promising producer of natural CTX (Khodaiyan, Razavi, & Mousavi, 2008). Therefore, the emulsions enrichment with CTX produced by *D. natronolimnaea* HS-1 can increase consumer's demand for consumption of these products.

Response surface methodology (RSM) is a useful statistical procedure used for multiple regression analysis by using quantitative data. This technique solves multivariate data which is found from appropriately designed experiments to solve multivariate equation simultaneously (Baş & Boyaci, 2007). Moreover, the main advantage of RSM is to reduce number of experimental trials needed to evaluate multiple variables and their interactions. Therefore, RSM is less laborious and time-consuming than the classical methods required optimizing a process (Cruz, Faria, Granato, Cavalcanti, & Walter, 2010; Myers & Montgomery, 2002).

The aim of this work was to optimize the droplet size and density properties of coconut oil (CO)-in-water emulsions containing bacterial CTX using RSM. In the present study, RSM was used. The optimization of emulsion components (GA, XG and CO) levels allows the manufacturers for the pre-formulation of optimum CTX emulsions with desirable physical characteristics.

2. Materials and methods

2.1. Chemicals and materials

Potassium sorbate was obtained from Chisso Co. (Tokyo, Japan). Sodium benzoate was provided by Fars Chemical Industry Co. (Shiraz, Iran) and food grade citric acid (anhydrous) was purchased from Kimia Gharb-Gostar Industry Co. (Kermanshah, Iran). GA was purchased from Merck KGaA (Darmstadt, Germany). XG was provided from Sigma–Aldrich (Oakville, ON, Canada). CO was purchased from local market. It was selected for emulsion production due to high saturated fatty acids and followed by reduction of oxidation problems. The used CO contained the following fatty acids (mol%): 7.9% C8:0, 6.5% C10:0, 45.6% C12:0, 18.3% C14:0, 8.7% C16:0, 2.9% C18:0, 7.1% C18:1 and 1.4% C18:2 as measured by gas chromatography of methyl esters.

2.2. Canthaxanthin extraction and analysis

The strain of bacterium *D. natronolimnaea* HS-1 (DSM 44860) used in this work to produce CTX was provided by Bioprocess Engineering Laboratory (BPEL), University of Tehran, Iran. Pigment extraction and analysis were performed as previously reported by Gharibzahedi, Razavi, & Mousavi (2013). Briefly, after preparing pre-culture in liquid yeast/malt agar medium, the inoculum was transferred into Erlenmeyer flasks containing 25 g/l beet root molasses and 10 g/l yeast extract. Finally, the flasks to produce CTX were incubated at 180 rpm and 28 ± 2 °C for 6 days. Then, aliquots (10-ml) of cultures were taken from the bioreactor and centrifuged at 7500 × *g* for 7.5 min. The produced supernatant was collected. Then, the cell pellets were washed twice with physiological water (NaCl; 9 g/l in deionized water) and centrifuged again. These cells were resuspended three times in 3 ml of pure ethanol by vortexing for 5 min and centrifuged again to extract the pigment. A water bath (45 °C) was also used to completely extract the pigments. The carotenoid extracts subsequently filtered through a 0.2 μm hydrophobic fluorophore membrane (Sigma–Aldrich Co., USA). Individual carotenoids were analyzed according to the modified method of Razavi, Blanchard, & Marc (2006) using a Knauer (Berlin, Germany) HPLC system on a Lichrospher 100 RP-18 silica column (5.0 mm, 250 mm × 4 mm) at 35 °C.

2.3. Preparation of emulsion samples

The emulsions were prepared according to the following formula: GA (6–10%, w/w), XG (0.1–0.3%, w/w), CO (3.5–6.5%, w/w), citric acid (0.4%, w/w), potassium sorbate (0.1%, w/w), sodium benzoate (0.1%, w/w) and deionized water. CTX was also added to CO fraction. Because of CTX negligible solubility at room temperature, it is dissolved in hot CO at a constant ratio of 1:50 to add it to the O/W emulsions. According to the previous study (Gharibzahedi, Razavi, et al., 2013), this ratio was an optimum level for the high solubility of CTX in CO

CTX is highly susceptible to thermal degradation, for that, the exposure time at high temperatures should be restricted. Authors previously examined various times and temperatures under vacuum conditions for achieving the lowest oxidation rate. The results showed that temperature of 97 °C and time 7.5 s at vacuum can lead to CTX high solubility with ideal oxidation level (Gharibzahedi, Razavi, & Mousavi, 2012). The peroxide value, anisidine value and total oxidation (Totox) value under these conditions were 0.22 mequiv. O_2/kg oil, 0.26 and 0.70, respectively. The main emulsion components (GA, XG and CO) were prepared for the optimization procedure based on a three-factor central composite design (CCD) (Table 1).

All emulsions containing microbial CX were prepared in two stages. The coarse emulsions were produced by Ultra Turrax (IKA T25 Digital, Germany) in 6000 × *g* for 10 min, and then further emulsified by using an 25 kHz ultrasonic homogeniser (UP200S, Hielscher Ultrasonics GmbH, Teltow, Germany) equipped with a 13-mm-diameter sonotrode probe made of titanium at a total nominal output power of 200 W. To prepare the water phase, citric acid, potassium sorbate and sodium benzoate were dispersed in deionised water (≈60 °C) using a blender (IKA-WERK, RW 20 DZM, Staufen, Germany). During continuous mixing, GA was gradually added to the deionized water (≈60 °C), and the solution was mixed for an extra 5 min to further facilitate hydration. To achieve full hydration, the mixture was kept at room temperature (23 ± 1 °C) overnight. XG solution was prepared separately by dissolving XG in deionized water with shaking and then mixed with the GA solution by using a high-speed blender (Gharibzahedi, Mousavi, et al., 2013). The dispersion of CTX in CO was also maintained overnight to rehydrate and then slowly added to the water phase to prepare an initial coarse emulsion. In the next stage, in order to produce the fine-disperse emulsions with small average droplet size and narrow particle-size distribution, the coarse emulsions were sonicated for 4 min (Gharibzahedi, Razavi, et al., 2013). By holding the vessel in a refrigerated water bath, the difference of temperature from initial coarse emulsions to final emulsion during emulsification was not more than 20 °C. At least two separate emulsions were prepared for each treatment. The emulsions were stored at 4 °C, and re-equilibrated to room temperature just before analysis.