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Optimization of mixed surfactants-based β-carotene nanoemulsions using response surface methodology: An ultrasonic homogenization approach

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ABSTRACT

In the present study, food grade mixed surfactant-based β-carotene nanoemulsions were prepared without using any co-surfactant. Response surface methodology (RSM) along with central composite design (CCD) was used to investigate the effect of independent variables (surfactant concentration, ultrasonic homogenization time and oil content) on response variables. RSM analysis results revealed that experimental results were best fitted into a quadratic polynomial model with regression coefficient values of more than 0.900 for all responses. Optimized preparation conditions for β-carotene nanoemulsions were 5.82% surfactant concentration, 4 min ultrasonic homogenization time and 6.50% oil content. The experimental values at optimized preparation conditions were 119.33 nm droplet size, 2.67*p*-Anisidine value and 85.63% β-carotene retention. This study will be helpful for the fortification of aqueous products with β-carotene.

1. Introduction

β-Carotene is a member of the carotenoid family, which is mainly found in fruits and vegetables. It provides a substantial proportion of vitamin A in the human diet because of its retinol precursor and higher conversion rate (Naves & Moreno, 1998). It is also useful in the prevention of numerous diseases, such as heart diseases, cataracts and cancer (Aherne, Daly, Jiwan, O'Sullivan, & O'Brien, 2010). Furthermore, it is also used in the food industry as a colorant and antioxidant (Hou et al., 2012). Therefore, the food industry is interested in its incorporation into food products to take advantage of the above-mentioned benefits. However, its incorporation into beverages and various other foods is challenging due to its poor water solubility, instability in heat, oxygen and light and appearance in crystalline state at ambient temperature (Mattea, Martín, Matías-Gago, & Cocero, 2009). To overcome this problem, β- carotene can be dissolved in oil or another suitable medium in oil in water emulsions before its incorporation into aqueous food products (Qian, Decker, Xiao, & McClements, 2012). Stability of β-carotene in oil in water emulsion depends on the composition of emulsion and environmental conditions, e.g. heat, surfactant, light, food systems, singlet oxygen and antioxidant addition (Hou et al., 2010). The most convenient way to incorporate β- carotene into food products is in a nanoemulsion based colloidal system.

Nanoemulsions are kinetically stable systems with mean radii of < 100 nm. Furthermore, these emulsions have higher stability, solubility and bioavailability due to their smaller particle size as compared to conventional emulsions (McClements & Rao, 2011). Nanoemulsions can be produced using high energy and low energy methods. During high energy methods, intense disruptive force is generated to mechanically break the oil phase into tiny droplets, which can be dispersed into the aqueous phase. These high energy methods (sonication, high pressure homogenization and microfluidization) are desirable for the food industry because we can prepare nanoemulsions by using lower surfactant to oil ratio as compared to low energy methods (Ozturk, Argin, Ozilgen, & McClements, 2014). Previously, some studies were carried out on the preparation of β-carotene nanoemulsions using low energy methods, microfluidization and high pressure homogenization but no study has been carried out on the preparation of nanoemulsions through the ultrasonic homogenization method. Hence, the present study was designed to investigate the suitability of ultrasonic homogenization for development of β-carotene nanoemulsions.

β-Carotene nanoemulsions prepared through the ultrasonic homogenization method were influenced by multiple variables during our laboratory experiments (unpublished data). So, there is a need for optimization of process or product in order to investigate the relationship between independent variables and response variables. Response

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surface methodology is an effective mathematical and statistical technique to investigate the effects of multiple independent variables and their interaction on response variables (Li, Wang, & Wang, 2017; Mehmood, Ahmad, Ahmed, & Ahmed, 2017). Hence, in our study, we have used RSM for optimization of emulsifying conditions.

The present study was designed to prepare mixed surfactant-based, co-surfactant free (due to irritation and toxic effects of co-surfactants) β-carotene nanoemulsions using an ultrasonication approach. After that, preparation conditions (surfactant concentration, homogenization time and oil content) for β-carotene nanoemulsions were optimized using RSM in order to obtain smallest droplet size, lower *p*-anisidine value and maximum β-carotene retention.

2. Material and methods

2.1. Materials

Tween 80 and soya lecithin were obtained from Sigma-Aldrich (St. Louis, USA). Purified β-carotene (powder form) was supplied by BASF (Lampertheim, Germany). Olive oil (refined, bleached and deodorized) was purchased from Hamza Vegetable Oil Refinery and Ghee Mills (Lahore, Pakistan). Double distilled water was used for the preparation of nanoemulsions and solutions.

2.2. Nanoemulsions preparation

Nanoemulsions were prepared by mixing 10% dispersed phase and 90% continuous phase. The dispersed phase was prepared by dissolving a pre-determined amount of β-carotene in olive oil (5.48–10.52%). The continuous phase consisted of double distilled water carrying pre-determined amount of surfactants (2.64–9.36%). These components were mixed with polytron (KRH-I, KONMIX, Shanghai, China) at 8000 rpm for 7 min to prepare coarse emulsions. For the preparation of nanoemulsions, these coarse emulsions were subjected to ultrasonic homogenization by using a 20 kHz sonicator (230VAC, Cole-Parmer, USA). Ultrasonic homogenization was performed by placing the tip horn (20 mm diameter) of the sonicator in coarse emulsions and applying ultrasonic powers for different times (2.98–8.02 min). The temperature of the emulsions was controlled by placing them in ice bath during homogenization. These nanoemulsions were stored at room temperature for further analysis.

2.3. Droplet size analysis

The droplet size of the nanoemulsions was measured by dynamic light scattering using nanotrac (Microtrac, Tri-Blue, USA). Nanoemulsion samples were diluted to 10% by using deionized water in order to avoid multiple scattering effects.

2.4. p-Anisidine value

p-Anisidine value is an important indicator of the stability of nanoemulsions. The oxidative stability of β-carotene nanoemulsions was determined according to the method of Mehmood et al. (2017). Firstly, 20 g of β-carotene nanoemulsions were incubated for one week at 50 °C. Then, 1 g of solution was dissolved in *n*-Hexane (HPLC Grade) and the absorbance of the solutions was measured using an UV-spectrophotometer at 350 nm. After that, 1 ml *p*-Anisidine reagent (prepared by dissolving 2.5 g of *p*-Anisidine in one litre of acetic acid) was added in 5 ml of solution and they were incubated for 10 min to allow their reaction. The absorbance of the fat solution was also determined as a blank in a reference cell. *p*-Aniside value was determined using Eq. (1):

$$p\text{–Anisidine Value} = \frac{25 \times (1.2\mathrm{A_{AR}}\text{–}\mathrm{A_{BR}})}{\mathrm{M}} \qquad (1)$$

where A_{AR} is the absorption of the solution after reaction, A_{BR} represents absorption before reaction and M denotes sample mass in grams.

2.5. β-Carotene retention

The concentration of β-carotene in nanoemulsions was determined after one week by a spectrophotometric method. Firstly, a 1 ml sample was extracted using a mixture of *n*-Hexane (3 ml) and ethanol (2 ml). After that, this mixture was shaken well and the hexane phase was removed. This extraction procedure was repeated two times more. At the end, all hexane phases were combined and their absorbance was measured through an UV- spectrophotometer at 450 nm after desired dilution with *n*-Hexane. The β-carotene concentration was determined using a standard curve prepared under similar conditions. Vitamin retention was calculated using Eq. (2):

$$\mathrm{VR_{BC}} = V_{\mathrm{BC,N}}/\mathrm{V_{BC,I}} \times 100 \qquad (2)$$

where VR_{BC} represents β-carotene retention, $V_{BC,N}$ is the concentration of β-carotene in the nanoemulsion and $V_{BC,I}$ indicates initial concentration of β-carotene (Yuan, Gao, Zhao, & Mao, 2008).

2.6. Experimental design

Response surface methodology was used to investigate the effect of independent variables, including surfactant concentration (X_1), ultrasonic homogenization time (X_2) and oil contents (X_3) on response variables, such as droplet size (Y_1), *p*-Anisidine value (Y_2) and retention of β-carotene (Y_3) in nanoemulsions. RSM design along with coded and uncoded levels is presented in Table 1. Central composite design (Five levels) and quadratic model was used to design this experiment. Twenty treatments, including six axial points, eight fractional factorial points and six central points were randomly performed according to CCD, which is summarized in Table 1. Real levels of independent variables were coded according to Eq. (3);

$$\mathrm{Z}= Z_0\text{––}\mathrm{Z_C}/\Delta Z \qquad (3)$$

where Z and Z_0 indicate coded and real levels of independent variables, respectively. ΔZ represents step change while Z_C indicates actual value at the central point. The specific equations for each independent variable were derived from the above equation to code their actual values. Specific equations for surfactant concentration (X_1), ultrasonic homogenization time (X_2) and oil contents (X_3) are mentioned in below Eqs. (4)–(6).

$$z_1 = (\mathrm{MS}\text{–}6)/2 \qquad (4)$$

$$z_2 = (\mathrm{HT}\text{–}5.5)/1.5 \qquad (5)$$

$$z_3 = (\mathrm{OC}\text{–}8)/1.5 \qquad (6)$$

where MS, HT and OC represent surfactant concentration, homogenization time and oil contents, respectively.

A second order polynomial equation was used to indicate the predicted responses (droplet size, *p*-Anisidine value and retention of β-carotene) as a function of an independent variable as follows (Eq. (7)):

Table 1
Independent variables and their corresponding levels for β- Carotene nanoemulsion.

Independent variable	Symbol	Coded levels				
		−α	−1	0	+1	+α
Surfactant Concentration (%)	X_1	2.64	4	6	8	9.36
Homogenization Time (min)	X_2	2.98	4	5.5	7	8.02
Oil Content (%)	X_3	5.48	6.5	8	9.5	10.52