



Application of image processing to assess emulsion stability and emulsification properties of Arabic gum

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ARTICLE INFO

Article history:

Received 10 June 2014

Received in revised form 20 February 2015

Accepted 9 March 2015

Available online 20 March 2015

Keywords:

Emulsion stability

Images processing

Arabic gum

RSM

ABSTRACT

This paper focuses on the development of an effective methodology to determine the optimum levels of independent variables leading to maximize stability of O/W emulsions containing Arabic gum, as a natural emulsifier and stabilizer. Response surface methodology (RSM) was employed to determine the effect of Arabic gum content (2%, 5%, and 8% (w/w)), homogenization time (5, 12.5, and 20 min) and storage temperature (4, 22, and 40 °C). Image processing was used to determine emulsion stability based on responses including creaming index, centrifugal stability, viscosity, color parameters, and D_{32} and D_{43} indices. For each response, a second-order polynomial model with high coefficient of determination (R^2) values ranging from 0.95 to 0.989 was developed using multiple linear regression analysis. The optimization results showed that the overall optimum region with the highest stability was found to be at the combined levels of 5.81% (w/w) Arabic gum content, 5 min homogenization time, and 22 °C for storage temperature.

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1. Introduction

An emulsion can be simply defined as “a system comprised of two immiscible liquids, one of which is dispersed as droplets (the dispersed or internal phase) throughout the other (the continuous or external phase)” (Jafari, Assadpoor, He, & Bhandari, 2008). Food emulsions are usually classified as either oil-in-water (O/W) or water-in-oil (W/O) depending on whether the oil phase forms the droplets or the continuous phase, respectively (Kim, Decker, & Julian McClements, 2006). Emulsions are thermodynamically unstable; with time they tend to breakdown into their constituent oil and aqueous phases. The term ‘emulsion stability’ therefore refers to the ability of an emulsion to resist this breakdown, as indicated by growth in average size of droplets or change in their spatial distribution within the sample.

The physicochemical properties and stability of the emulsions formed are largely determined by many factors such as emulsifier concentration, the volume fraction of the oil phase to the aqueous phase, method of mixing, droplet size, viscosity, surface tension and temperature as well as the homogenization and other processing conditions employed (Kim et al., 2006). Several physicochemical mechanisms contribute to the instability of

an O/W emulsion, including gravitational separation (creaming), coalescence, flocculation (partial) coalescence, and Ostwald ripening. These processes are closely interrelated (Birdi, 2008; Dickinson, 2009; Huang, Kakuda, & Cui, 2001; Mollet & Grubenmann, 2008). The stability and the emulsion formation become easier when using an emulsifier, which would be adsorbed at the interface between oil and water and can lower the interfacial tension and prevent coalescence of droplets by increasing repulsion forces between droplets (McClements, 1998; Perrier-Cornet, Marie, & Gervais, 2005).

Polysaccharides are good stabilizing agents because of their hydrophilicity, highly-branched structure, and high molecular weight that impart gelling and thickening properties to them and thereby provides a macromolecular barrier against destabilizing mechanisms by increasing the viscosity of the aqueous phase and slowing flocculation and coalescence between dispersed droplets. Arabic gum (Acacia) is an extremely important and commonly employed emulsifying agent which maintains its function effectively at different conditions (low pH, high ionic strength, etc.) (Birdi, 2008; Dickinson, 2009; Jafari, Beheshti, & Assadpoor, 2012; Larsson & Friberg, 1990; Soleimanpour, Koocheki, & Kadkhodaei, 2013). Arabic Gum is a complex blend of natural polysaccharides composed of three components differing in molecular size and protein content. These are commonly referred to as arabinogalactans (AG), arabinogalactan proteins (AGP), and glycoproteins (GP). Between them, it has been well established that AGP complex is the main component responsible for GA ability to stabilize

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emulsions. Detailed investigation on the structure, molecular weight parameters and emulsification performance of Arabic gum has been published elsewhere (Al-Assaf, Sakata, McKenna, Aoki, & Phillips, 2009; Castellani et al., 2010; Mahendran, Williams, Phillips, Al-assaf, & Baldwin, 2008). Also, the role of Arabic gum as a native gum in emulsion stability and optimum emulsification condition has been studied in many works (Charoen et al., 2011; Jafari et al., 2012; Mirhosseini, Tan, Hamid, & Yusof, 2008; Wang, Wang, Li, Adhikaric, & Shid, 2011).

Several works have been carried out to investigate and control instability mechanisms and prolong shelf life of products (Chanamai & McClements, 2001a; Chantrapornchai, Clydesdale, & McClements, 2001; Dickinson, Radford, & Golding, 2003; Dickinson & Ritzoulis, 2000; Mirhosseini et al., 2008; Pichot, Spyropoulos, & Norton, 2010; Soleimanpour et al., 2013). The applied methods for evaluating emulsion stability include creaming or sedimentation processes, microscopic observations, droplet size analysis, freeze-etching, rheology measurements, self-diffusion NMR, etc. (Scherze, Knöfel, & Muschiolik, 2005).

Recently, computer vision employing image processing techniques has been developed rapidly, which can quantitatively characterize complex size, shape, color, and texture properties of foods in order to provide objective, rapid, non-contact, and non-destructive quality evaluations (Du & Sun, 2004; Zheng, Sun, & Zheng, 2006). Basically, an image processing system consists of a digital or video camera for image acquisition, standard settings illuminants, and a computer software for image analysis (Li, Wang, & Gu, 2002; Pedreschi, León, Mery, & Moyano, 2006; Sun, 2004). Previous works dealt with emulsions pointed out that light microscopy and image processing can be used successfully for the analysis of emulsions (Freire, Dias, Coelho, Coutinho, & Marrucho, 2005; Meijer, Abbes, & Hansen, 2001; Scherze et al., 2005; Schuster et al., 2012; Silva, Rocha-Leão, & Coelho, 2010). However, a detailed study on emulsion stability using image processing has not yet been done. Therefore, the objective of this study was to maximize emulsion stability by modeling the possible relationships between the responses and independent variables. In addition, our aim was to determine the stability of emulsions by image processing in comparison with conventional methods.

2. Materials and methods

2.1. Materials

Arabic gum (*Acacia senegal*, molecular mass ~380,000) was provided by Scharlau (Germany), and sodium dodecyl sulfate (SDS) was purchased from the Merck Company (Germany). Edible sunflower oil (Ladan, Iran) acquired at a local market (22.8% monounsaturated fatty acids, 65.5% polyunsaturated fatty acids, 12% saturated fatty acids, and containing citric acid and TBHQ as artificial antioxidant). Distilled and deionized water, prepared in our laboratory, was used for the preparation of all solutions and emulsions. All other chemicals used in this study were of analytical grade and purchased from chemical suppliers.

2.2. Experimental design for response surface methodology

Response surface methodology (RSM) was employed to investigate the variation of stability with respect to operating parameters including emulsifier (Arabic gum) concentration, storage temperature and homogenizing time. The composition of three variables was designed by central composite design (CCD) approach. CCD is a 2^k factorial design with star points and central points. The coded variables and their concentration are shown in Table 1. Twenty experimental settings and three central points were generated with three factors and three levels using Design-Expert 6.0.8 (StatEase,

Table 1

Coded levels for independent variables used in experimental design for stability of emulsions by gum Arabic.

Variables	Coded X_i	Coded level		
		–1	0	1
Concentration of gum Arabic (% w/w)	X_1	2	5	8
Homogenizing time (min)	X_2	5	12.5	20
Storage temperature (°C)	X_2	4	22	40

Inc., Minneapolis, MN). The numerical and graphical optimizations were also performed by the same software. Experiments were randomized in order to minimize the effects of unexplained variability in the observed responses due to extraneous factors. The center point in the design was repeated six times to calculate the repeatability of the method. A second-order polynomial model was constructed to estimate each response (Eq. (1)):

$$y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j>1}^k \beta_{ij} X_i X_j + e \quad (1)$$

where y is the estimated response (dependent variable), b_0 the model constant, b_i the linear effect coefficient, b_{ii} the quadratic effect coefficient, b_{ij} the interaction coefficient for two factors, x_i , x_j the independent variables, e the error, k the number of variables considered, i and j the codified factors of the system (Baş & Boyacı, 2007; Montgomery, 2001; Taherian, Britten, Sabik, & Fustie, 2011).

2.3. Emulsion preparation

Prepared emulsions were oil in water emulsions containing Arabic gum as an emulsifier and were prepared according to the modified method of Jafari et al. (2012). Arabic gum was dissolved in distilled water (60°C) and for complete hydration, it was kept in water bath (60°C) and prepared 1 day before emulsification. The sunflower oil was added drop-wise into aqueous phase while stirring with a homogenizer (Heidolph Silentcrusher, Germany) at 8000 rpm for 5 min in order to make pre-emulsions. Final emulsion was prepared by homogenization at 12,000 rpm for 5, 12.5, and 20 min.

2.4. Analysis of emulsion stability

2.4.1. Creaming stability measurement

A 20 ml freshly prepared emulsion was transferred into a test tube, capped, and stored at 4, 22, and 40°C. Creaming of emulsions was monitored after 1, 7, 14, 21, and 28 days storage. During storage, some of the samples separated into an opaque cream layer at the top and a transparent serum layer at the bottom. The total height of the emulsion (HE) and the height of the serum layer (HS) were then measured. The creaming index (CI) was expressed as the ratio of the height of the serum layer to the total height of the emulsion in the tube (Eq. (2)): (Koocheki, Kadkhodaei, Mortazavi, Shahidi, & Taherian, 2009; Nikiforidis & Kiosseoglou, 2007)

$$CI = \frac{HS}{HE} \times 100 \quad (2)$$

2.4.2. Centrifuge stability measurement

Centrifugation method for emulsion stability (ES), was determined by centrifugation 5 ml of emulsions at 3500 rpm for 15 min. ES was calculated as:

$$ES = \frac{f_{ev}}{i_{ev}} \times 100 \quad (3)$$

where f_{ev} is the final emulsion volume, and i_{ev} is initial emulsion volume (Sciarini, Maldonado, Ribotta, Pérez, & León, 2009).

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