Food Hydrocolloids 37 (2014) 49–59

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Assessment of emulsifying ability of almond gum in comparison with gum arabic using response surface methodology

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ARTICLE INFO

Article history:
Received 8 March 2013
Accepted 15 October 2013

Keywords:
Almond gum
Gum arabic
Emulsification
High pressure homogenization
Response surface methodology
Interfacial tension

ABSTRACT

The emulsifying properties of almond gum from *Prunus dulcis* were assessed in comparison with gum arabic from *Acacia senegal*.

Interfacial properties were preliminary evaluated by pendant drop method, while emulsifying ability was quantified in terms of mean droplet size of O/W emulsions prepared at different intensity levels of high pressure homogenization as well as of stability of resulting optimized emulsions.

Response surface methodology (RSM) was used to determine the optimum emulsification conditions for minimum mean emulsion droplet size. Homogenization pressure (100–300 MPa), number of passes (1–10 passes) and gum concentration (5, 7.5 and 10% w/w) were the factors investigated. Experiments were designed according to a three-level, three-variable Box–Behnken design (BBD), and a second-order polynomial model was developed for the response variable using multiple linear regression analysis, which resulted to be very accurate both for almond gum ($R^2 = 0.979$) and for gum arabic ($R^2 = 0.993$).

Results showed that almond gum exhibited good emulsifying abilities, yet different from gum arabic. The measured interfacial properties of almond gum showed slower dynamics of adsorption and reorganization at the oil-water interface. Coherently, the optimum emulsification conditions determined by RSM required for almond gum a lower emulsifier concentration (5.7%) than for gum arabic (8.4%), but the use of gum arabic allowed for a smaller mean droplet size at lower intensity of high pressure homogenization treatment. Remarkably, the stability of 10% oil emulsions using almond gum as emulsifier was comparable to those using gum arabic, for gum concentrations in excess of 5%.

1. Introduction

Among food biopolymers, only a few exhibit a significant emulsification ability, which has been attributed to the presence of a proteinaceous moiety, imparting surface active properties (Dickinson, Murray, Stainsby, & Anderson, 1988; Randall, Phillips, & Williams, 1989).

In recent years, a growing interest was observed for new sources of biopolymers to be used in the food industry (Dickinson, 2003; Garti, Slavin, & Aserin, 1999; Marcotte, Hoshahili, & Ramaswamy, 2001; Vilela & Ravetta, 2005). Some food biopolymers, such as gum exudates, mainly composed of polysaccharides, exhibit specific interfacial properties, which enable their application as emulsifying and stabilizing agents in the formulation of stable emulsions, especially thanks to their ability to form structured interfacial films (Orozco-Villafuerte, Cruz-Sosa, Ponce-Alquicira, & Vernon-Carter, 2003). Many plants have been chemically analyzed as potential sources of gum exudates, such as *Acacia senegal*, from which the famous gum arabic is obtained (Al-Assaf, Phillips, Aoki, & Sasaki, 2007; Dickinson et al., 1988; Mothé & Rao, 1999). Among all gum exudates, gum arabic has the highest commercial value due to its widespread application in food, pharmaceutical and cosmetic industries (Whistler & Bemiller, 1993). During the homogenization process, gum arabic adsorbs at the surface of the freshly formed fine droplets and forms a relatively thick and negatively charged interfacial layer around them, preventing their aggregation due to steric hindrance and electrostatic repulsion (Chanamai & McClements, 2002).

The process of formation of a stable oil-in-water emulsion made of fine droplets is influenced by several parameters, such as the

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0268-005X/$ – see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.foodhyd.2013.10.009

properties and concentration of the emulsifier and of the oil phase, pH and ionic strength of the aqueous solution (Donsì, Annunziata, Vincensi, & Ferrari, 2012, Donsì, Sessa, & Ferrari, 2012, Donsì, Wang, & Huang, 2011), as well as the type and the operating conditions of equipments used for emulsification, such as high-shear mixer, colloid mill or high-pressure valve homogenizer (Donsì, Sessa, et al., 2012). When many operating parameters and their reciprocal interactions affect the final outcome of a process, response surface methodology (RSM) is an effective tool for optimizing their choice and selection. It enables the minimization of the number of experimental trials required and the evaluation of the interactions between multiple parameters. RSM is a collection of mathematical and statistical techniques for analyzing the influence of dependent and independent variables on the response of a process, based on the response surface formed from the result of the factorial design tests (Myers & Montgomery, 2002). RSM has been successfully applied for optimizing conditions in food research (Ibanoglu & Ainsworth, 2004; Varnalis, Brennan, MacDougall, & Gilmour, 2004).

In this work, a novel gum exudates from *Prunus dulcis*, almond gum (AL), is compared with the well established gum arabic (GA), an exudate from *A. senegal* trees, in terms of their emulsifying abilities. Almond gum is a gum copiously exuded from the trunk, branches and fruits of *P. dulcis* trees, after mechanical injury and/or infection by microorganisms. Previous studies have demonstrated that almond gum is mainly composed of 92.36% of polysaccharides (dry weight basis) with the major sugar composition being arabinose (46.83%), galactose (35.49%) and uronic acid (5.97%), along with low levels of protein (2.45%) (Mahfoudhi, Chouaibi, Donsì, Ferrari, & Hamdi, 2012).

The comparison of the two gums is based on the characterization of their respective interfacial properties and on the evaluation of their emulsifying abilities, measured in terms of mean droplet size of n-hexadecane in water emulsions produced by high pressure homogenization and their resulting physical stability under accelerated ageing. In consideration of the fact that the two gums require different operating conditions for the production of fine emulsions (pressure and number of passes, gum concentration), the comparison is carried out under the optimal conditions, as determined by RSM.

2. Materials and methods

2.1. Materials

Gum arabic from *A. senegal* trees was supplied by CNI (Colloides Naturels International, Rouen, France). Almond gum from *P. dulcis* trees was manually collected and dried at ambient temperature. Subsequently, it was purified according to the following steps: (a) it was dissolved in distilled water and stirred overnight using a magnetic mixer (25 °C, 500 rpm); (b) the solution was filtered to remove impurities and insoluble hydrogels; (c) the filtrate was further dialyzed against deionized water for 48 h; (d) residual water was removed by lyophilization using a freeze dryer ALPHA 2-4/LSC (Martin Christ GmbH, Germany) to collect a fine powder of polysaccharides. n-hexadecane was purchased from Sigma Aldrich (St Louis, MO, USA). All other solvents and reagents were of analytical grade.

2.2. Preparation of almond gum and gum arabic aqueous solutions

Aqueous solutions of almond gum (AL) and gum arabic (GA) were prepared at different concentrations (5, 7.5 and 10% (w/w)). Desired amounts of gum powder were dissolved in deionized water containing 0.02% (w/w) sodium azide and stirred overnight to ensure complete dissolution. Sodium azide was used as a protective agent against the microbial growth. The pH of the AL and GA aqueous solutions was adjusted to 5.0 using 0.1 M HCl or 0.1 M NaOH, as required.

2.3. Emulsions production

Oil-in-water emulsions were prepared by mixing 10% (w/w) n-hexadecane as oil phase with 90% (w/w) aqueous phase containing 5, 7.5 or 10% (w/w) almond gum or gum arabic as emulsifier. Primary emulsions were produced by high shear mixer (HSM) using an Ultra Turrax T25 (IKA Werke GmbH & Co., DE) equipped with a S25 N18 G rotor operated at 20,000 rpm for 5 min at 4 °C. Further processing of primary emulsions was used to produce secondary emulsions of very fine size, by high pressure homogenization (HPH) in a Nano DeBEE 45 electric bench-top laboratory machine (Bee Int., USA), as previously described in details (Spigno et al., 2013). The operating conditions varied between 100 and 300 MPa pressure levels, and the number of homogenization passes varied from 1 to 10. The inlet temperature was set at 4 °C and the outlet temperature, which was increased due to inherent heating (about 0.17 °C/MPa), was rapidly reduced in a heat exchanger to 4 °C placed immediately downstream of the homogenization valve.

2.4. Droplet size measurements

A photon correlation spectrometer (HPPS, Malvern Instruments, Malvern, UK) was used for the droplet size measurement of the HSM and HPH treated emulsions, whose characteristic size was always comprised in the instrument sensitivity range (1–6000 nm). The droplet size distribution was characterized in terms of the mean droplet size (Z-average diameter), which was determined by cumulant analysis of the intensity–intensity autocorrelation function $G(q,t)$, as previously described (Donsì, Wang, Li, & Huang, 2010) and polydispersity index (PDI). Prior to measurements, the samples were diluted with bidistilled water to a suitable concentration (usually a 1:100 dilution was applied).

2.5. Dynamic interfacial tension measurements

The interfacial tension was measured at the oil-water interface, by means of the pendant drop method, as previously described by Donsì, Senatore, Huang, and Ferrari (2010), using n-hexadecane as oil and an aqueous solutions of GA or AL as water phase at the concentration of 0.5% (w/w) and of 5% (w/w). In addition, the effect of HPH treatment on the morphology and functionality of the tested gums was assessed by comparing the interfacial tension of 5% (w/w) aqueous solutions of GA or AL before and after a HPH treatment (10 passes at 200 MPa). A CAM200 apparatus (KSV Instruments, Finland), consisting of an experimental cell, an illuminating, and viewing system to visualize the drop as well as a data acquisition system, was used to determine the interfacial tension from the pendant drop profile. Images of the drop are captured and digitalized, and the drop contour is extracted for the determination of the radius of curvature at the apex necessary for the calculation of the interfacial tension. Surface tension is automatically determined via software (CAM software, supplied with the instrument) by fitting the shape of the drop (in a captured video image) to the Young–Laplace equation which relates interfacial tension to drop shape, as previously described in details (Donsì, Sessa, et al., 2012). Equation (1) reports the Young–Laplace equation, where Φ is the angle made by the tangent at the point (X, Z), s is the linear distance along the drop profile, $d\Phi/ds$ corresponds to the radius of curvature at the point (X, Z) and β is the shape parameter, given by equation (2), where g is the gravitational constant, γ is the effective density