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Stabilization of W/O/W multiple emulsion loaded with *Hibiscus sabdariffa* extract through protein-polysaccharide complexes

Sandra Pimentel-Moral[a,*], Javier M. Ochando-Pulido[b], Antonio Segura-Carretero[a], Antonio Martinez-Ferez[b]

[a] *Department of Analytical Chemistry, University of Granada, 18071 Granada, Spain*
[b] *Chemical Engineering Department, University of Granada, 18071 Granada, Spain*

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ABSTRACT

In this work, a hydrophilic anthocyanidin-rich extract from *Hibiscus Sabdariffa (H. sabdariffa)* was vehiculized in the inner phase of a water/oil/water emulsion and its physical stability was improved by means of the reinforcement of both interfaces, the oil-water and the water-oil one, with whey protein concentrate (WPC)-arabic gum (AG) complexes. To this end, the main critical parameters involved on destabilization mechanisms for emulsions have been considered, e.g. the active ingredient concentration, the emulsifier amount and ratio, the biopolymers concentration and ratio (WPC:AG), and the pH value during emulsification. Stable and constant values of average droplet volume diameter ($d_{4,3}$) around 6.47–8.54 μm after 30 d of storage at room temperature were obtained for multiple emulsions containing 20 and 10 g/100 g of extract concentration, respectively. The pH was found to play an important role in the long-term stability. Indeed, the higher long-term stability parameters were achieved at pH of 4.5, value in which the WPC and GA result in maximum complex formation. Thus, the stabilization of water/oil/water multiple emulsions containing *H. sabdariffa* extract through WPC and AG complexes was confirmed to be a potential strategy to improve physical stability over time.

1. Introduction

Hibiscus sabdariffa (Malvaceae family) is a tropical plant commonly used in the preparation of herbal drinks, hot and cold beverages, because of its antioxidant function in humans. Besides this, recent studies have shown that these extracts can help reduce some diseases as diabetes mellitus, cancer, dyslipidemia and hypertension (Fernandez-arroyo, Camps, Menendez, & Joven, 2015; Gurrola-Diaz et al., 2010; Mohamed, Shing, Idris, Budin, & Zainalabidin, 2013). These functional characteristics described for *H. sabddariffa* result from its composition since this plant contains different antioxidant compounds, mainly flavonoids and anthocyanins (Cid-Ortega & Guerrero-Beltran, 2015). The antioxidant activity of this extract has been investigated in several studies. In this sense, Frank et al. (2012) carried out a randomized study to evaluate the impact of *H. sabdariffa* extract on the systemic antioxidant status. These researchers measured the antioxidant activity through the ferric reducing ability of plasma (FRAP), and showed that this extract can help to improve the systemic antioxidant potential and decrease the oxidative stress in humans. In addition, after its consumption, urinary hippuric acid excretion was reported to increase due to a high microbial biotransformation of the ingested extract.

However, the proven bioactivity for *H. sabdariffa* extracts is very short-term due to rapid oxidation or insufficient gastric residence time, low permeability and/or solubility within the gut. Indeed, polyphenols are very unstable under typical food processing and storage conditions (e.g., temperature, oxygen, light exposure, etc), or through the gastrointestinal tract (e.g., pH, enzymes, interaction with other nutrients, etc) leading to partial or even total loss that restricts their application in products for human consumption (Munin & Edwards-Lévy, 2011). In this regard, Aurelio, Edgardo, and Navarro-Galindo (2008) showed that the bioactive components found in *H. sabdariffa* may be degraded during cooking process, losing many of their health properties.

Therefore, in order to establish a vehicle to incorporate antioxidants from *H. sabdariffa* extracts in food matrices it is necessary to reduce or prevent the degradation of flavonoids and to develop products capable to protect and deliver high enough concentration of these compounds, improving their bioactivity in parallel.

Within this context, multiple emulsions are a promising technology to accomplish this challenge and entrap/protect the bioactive ingredients of this extract. Water-in-oil-in water ($W_1/O/W_2$) emulsions consist of small water droplets contained within larger oil droplets that are themselves dispersed within a water continuous phase. Consequently, there are two

* Corresponding author.
E-mail addresses: spimentel@ugr.es (S. Pimentel-Moral), jmochandop@ugr.es (J.M. Ochando-Pulido), ansegura@ugr.es (A. Segura-Carretero), amferez@ugr.es (A. Martinez-Ferez).

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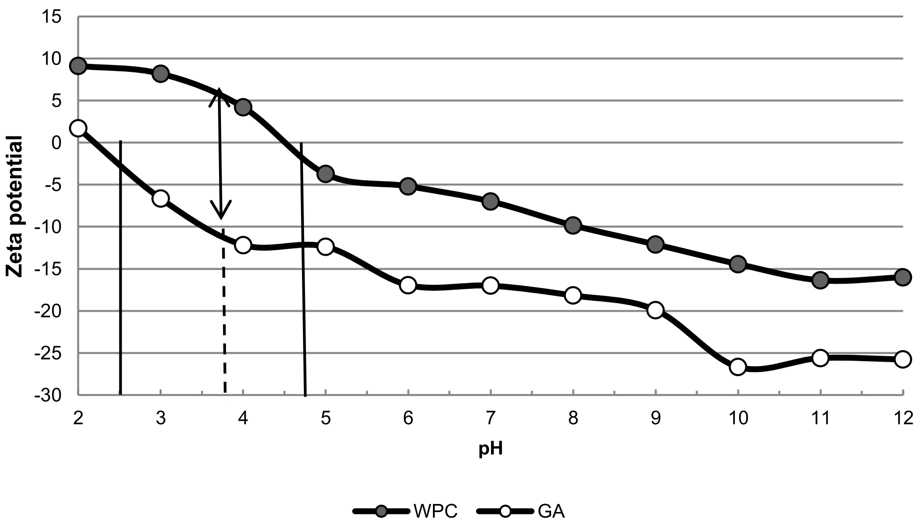

Fig. 1. Zeta potential as a function of pH for whey protein concentrate (closed symbols) and gum arabic (open symbols) solutions. Zeta potential was measured in mV.

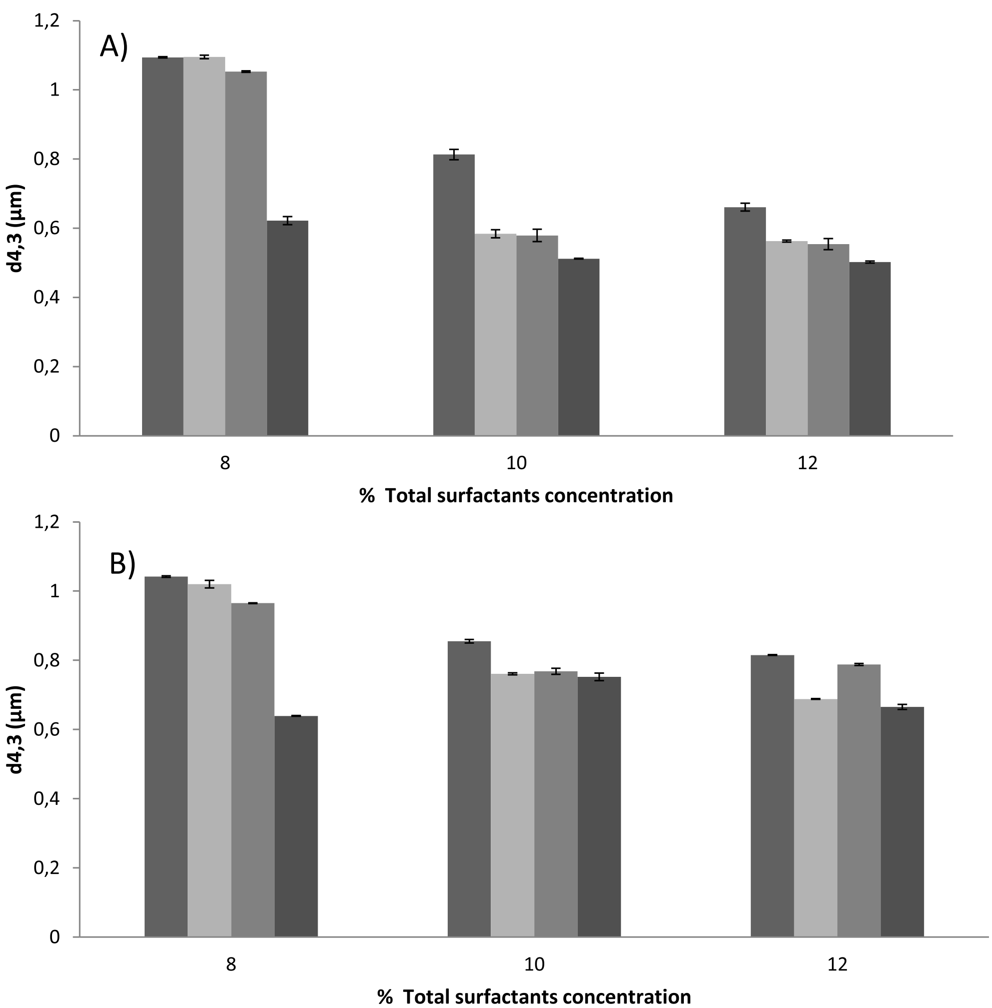

Fig. 2. Volume mean diameter ($d_{4,3}$) after 30 d at room temperature as a function of the mass ratio (respectively) and surfactants concentration. A) 20 g/100 g *H. sabdariffa* extract, B) 10 g/100 g *H. sabdariffa* extract.

different interfaces (W_1-O and O-W_2) that need to be stabilized by an oil-soluble emulsifier and a water-soluble emulsifier, respectively. Thereby, this structured delivery system can be used for protection, encapsulation and release of hydrophilic and hydrophobic active compounds (McClements, 2015; Pimentel-Moral, Verardo, Robert, & Segura-Carretero, Martínez-Férez, 2016). Multiple emulsions have already demonstrated to be able to protect and gradually release bioactive compounds. For example, Jiménez-Alvarado, Beristain, Medina-Torres, Román-Guerrero, and Vernon-Carter (2009) studied the encapsulation of ferrous bisglycinate in the inner aqueous phase of a $W_1/O/W_2$ emulsion stabilized by protein-polysaccharide complexes with good stability, encapsulation efficiencies, protection against oxidation, and slow release rates. More recently, Akhtar, Murray, Afeisume, and Khew (2014) encapsulated successfully rutin and anthocyanins within the internal aqueous phase of W/O/W multiple emulsions using spinning disc reactor technology, giving an encapsulation efficiency of > 80%.