Food Hydrocolloids 87 (2019) 382–393

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Characterization of prebiotic emulsions stabilized by inulin and β-lactoglobulin

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ARTICLE INFO

Keywords:
Inulin
Emulsion
Interface
Rheology
β-lactoglobulin

ABSTRACT

Inulin is a prebiotic ingredient that is being increasingly used in food formulations as fat replacer. Even if no significant surface activity has been reported for this natural polysaccharide, it can be used as ingredient in Oil in Water (O/W) emulsions that would benefit from its potential thickening effect of the continuous phase. In this manuscript, a well-characterized milk protein (β-lactoglobulin) was used at 0.5 % wt. content as emulsifier, while inulin was included in different concentrations from 0 to 10 % wt., in pressure-homogenized O/W emulsions (5/95). Zeta-potential measurements suggest the absence of important interactions between the protein and the polysaccharide in the continuous phase, observing an increase in the viscoelastic properties and viscosity of these pseudoplastic systems as inulin content is higher. In spite of no detecting any interfacial activity for inulin, its presence seems to favor the kinetics of protein adsorption, which might be attributed mainly to a thermodynamical incompatibility phenomena between both biopolymers. At high enough inulin content, the potential existence of protein-polysaccharide complexes become more probable, which may hinder protein adsorption, leading to a diminution of the steady-state interfacial pressure and an important reduction in interfacial dilatational modulus. The study of the droplet size distribution of the emulsions along storage time reveal how the bimodal distributions obtained keep constant, pointing out the stability of the emulsions prepared, a fact that is further supported by backscattering measurements along time. Emulsions including a prebiotic like inulin have an enormous potential in the food industry (e.g. smoothies).

1. Introduction

An increasing interest in healthy foods has led to low-fat or fat-free formulations that include fat replacers instead (Sandrou & Arvanitoyannis, 2000). Thus, the use of polysaccharides, such as pectin, starch, kefiran, inulin, xanthan gum or carrageenan, as fat substitutes is common nowadays in the food industry, due to their thickening and gelling properties and their healthy properties (Warrand, 2006). Emulsions are thermodynamically unstable systems (McClements, 2015), but kinetically stable emulsions may be achieved through the addition of proper emulsifiers and thickening agents. Emulsifiers are surface active substances that can be adsorbed on the oil droplet surface to produce an interfacial layer, which should be thick and compact enough to confer electrostatic and steric stabilization against several destabilization processes (Tcholakova, Denkov, Ivanov, & Campbell, 2006). On the other hand, thickening agents improve the stability through an increase in the viscosity of the continuous phase and their yield stress, as well as inhibiting the droplets mobility (Dickinson, 2003; Paraskevopoulou, Boskou, & Kiosseoglou, 2005). Thus, polysaccharides are one of key components in the formulation of commercial emulsions in order to control their shelf-life and organoleptic properties. Moreover, interactions between proteins and polysaccharides may exert an important role in the structure and stability of lots of processed food products (Tolstoguzov, 1991), as polysaccharides molecules may link protein molecules through covalent bonding resulting in permanent conjugates (Benichou, Aserin, Lutz, & Garti, 2007; Rodríguez Patino & Pilosof, 2011). They are normally found together in the formulation of food products, making their mixtures characterization extremely important (Norton & Frith, 2003). These protein-polysaccharide interactions may modify the functional properties of food proteins, like their interfacial activity, solubility, foaming or emulsifying properties, highlighting the role of those interactions on the relation between structure and properties of food systems. In this sense, the control or manipulation of the protein-polysaccharide interactions is very important for the development of novel products and processed food (Tolstoguzov, 1991).

Inulin or α-D-glucopyranosyl-[β-D-fructofuranosyl](n-1)-D-fructofuranoside is a natural polysaccharide considered as dietary fibre

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https://doi.org/10.1016/j.foodhyd.2018.08.024
Received 15 May 2018; Received in revised form 8 August 2018; Accepted 15 August 2018
Available online 19 August 2018
0268-005X/ Published by Elsevier Ltd.

frequently used as food ingredient (Franck, 2002). Additionally, some studies have shown it has remarkable biological effects, being a strong complement pathway activator and having anticancer (Cooper & Carter, 1986; Korbelik & Cooper, 2007) and immunomodulatory properties (Cooper & Steele, 1988, 1991; Cooper, McComb, & Steele, 1991; Silva, Cooper, & Petrovsky, 2004), and being able to be used also as functional and prebiotic ingredient (Kolida, Tuohy, & Gibson, 2002; Roberfroid, 2002). Moreover, inulin cannot be neither absorbed in the small intestine nor hydrolysed because of its chemical structure, although is widely fermented by bacteria in the large intestine (Adebola, Corcoran, & Morgan, 2014; Bot, Erle, Vreeker, & Agterof, 2004). Apart from its prebiotic character, some authors found an anticytotoxic and antigenotoxic effect of this polysaccharide. The use of inulin as fat replacer is promoted by its particular water solubility, as their hydroxyl groups are more prone to interact with water molecules than other functional groups present in its structure. This provides inulin the ability to form stable gels with water at concentrations around 13–50% (Castelli et al., 2008; Kim, Faqih, & Wang, 2001). The inulin gel network is composed by clusters of aggregated crystallites, providing analogous properties to those of a network of fat crystals in oil. Thus, inulin has been recognised as an attractive constituent for structuring low fat food products (Bot et al., 2004; Petrovsky, 2010) with good organoleptic properties (BeMiller, Steinheimer, & Allen, 1967; Castelli et al., 2008).

Milk proteins, like β-lactoglobulin or β-casein, show excellent emulsifying and emulsion stabilising properties, being commonly used in the formulation of food emulsions. Previous studies have focused on the potential interactions between some milk proteins and inulin (Schaller-Povolny & Smith, 2002), suggesting in some specific cases the formation of complexes between β-lactoglobulin and inulin. These complexes were found after heat treatment and explained on basis of either hydrophobic interactions or covalent Maillard-type interactions (Schaller-Povolny & Smith, 2002).

Due to prebiotic properties and the wide functional properties of inulin (thickener, gelling agent, sugars and fat substitute, etc.), its use as an ingredient in the formulation of functional foods is increasing. No previous studies are found in the literature about the interfacial characterization of protein-inulin mixtures. In this sense, this contribution is focused on the interfacial, rheological and microstructural characterization of Oil in Water (O/W) emulsions stabilized by a typical milk protein (β-lactoglobulin 0.5 % wt.) and different contents of inulin (0–10 % wt.)(Franck, 2002). The physical stability of the food emulsions prepared was also studied, due to its high importance during transport and storage.

2. Materials and methods

2.1. Materials and sample preparation

2.1.1. Materials

β-lactoglobulin was provided as lyophilized powder (≥90%) (Sigma-Aldrich, United States), and inulin (degree of polymerization: 36; free glucose and fructose (≤0.05%)) used was obtained from chicory (I2255, Sigma-Aldrich, United States). Sunflower oil was purchased from a local store which was used as the oil phase without further purification in order to study the performance in a real interface (this oil is widely used in food industry). Some adsorption measurements were performed using purified oil (Florisil 60–100 mesh Aldrich) to check the effect that any surface active impurities present in the oil might have on the interfacial behaviour (Camino, Sanchez, Rodríguez Patino, & Pilosof, 2012). It was observed that, at the protein concentration used (0.5 % wt.), the removal of impurities did not significantly affect the interfacial tension evolution.

All ingredients used in this work were food grade.

2.1.2. Sample preparation

2.1.2.1. Phase continuous. Protein solutions were obtained dispersing the convenient amount of β-lactoglobulin in Millipore water (1 % wt.). Inulin was placed in Millipore water with continuous stirring until complete solubilization. The inulin content in these solutions was adjusted in order to obtain a final polysaccharide concentration of 2.5, 5, 7.5 and 10 % wt. in the continuous phase, obtained after mixing with the β-lactoglobulin solution at 1:1 ratio. No significant phase separation has been visually detected in those systems, neither at low or high inulin contents. Sodium azide was used as antimicrobial agent (0.02 % wt.). The pH of the continuous phases was measured, being always neutral (pH 7).

2.1.2.2. Emulsions. O/W emulsions were prepared first slowly adding sunflower oil to the aqueous continuous phase as they were homogenized with an Ultra-Turrax T25 Basic (Germany) by applying a rotational speed of 3500 rpm for 3 min. The different pre-emulsions were then forced to pass once through a high-pressure valve homogenizer EmulsiFlex-2000-C5 (Germany) at 70 MPa. Temperature was controlled along emulsification and was always lower than 35 °C. Final emulsions contained 5 % wt. sunflower oil and 95 % wt. continuous phase.

2.2. Methods

2.2.1. Zeta potential

A Dynamic Laser Light Scattering instrument (Zetasizer Nano-Zs, Malvern Instruments, United Kingdom) was used for zeta potential measurements, being assessed from the electrophoretic mobility of the particles. Samples were previously diluted 1:100 with Millipore water. The zeta potential was determined at 25 °C making ten readings per sample, and being reported as the average and standard deviation of measurements made on at least two samples.

2.2.2. Rheological properties

Shear rheology tests for both the continuous phases and the emulsions were performed using an AR 2000 rheometer (TA Instruments, USA). An aluminium low-inertia parallel plate geometry with a diameter of 60 mm and a gap of 1 mm was used for all the shear rheological tests.

In shear flow tests, shear rate was increased step-by-step over the chosen range of shear rates (0.1–100 s^{-1}), being a steady state obtained at each shear rate.

Small Amplitude Oscillatory Shear (SAOS) measurements were conducted in the same rheometer to obtain the rheological properties within the Linear Viscoelastic Region (LVR) for all the samples studied at frequencies between 0.1 and 100 Hz.

2.2.3. Interface pressure and dilatational properties

Dynamic interface pressure and dilatational measurements were carried out using an automatic pendant drop tensiometer (TRACKER, IT Concept, France), as previously described (Baeza, Carrera Sanchez, Pilosof, & Rodríguez Patino, 2005; Perez, Carrera, Sánchez, Santiago, & Rodríguez Patino, 2009; Rodríguez Patino, Rodríguez Niño, & Sánchez, 1999). The interfacial dilatational modulus (E) was obtained through a periodic sinusoidal interfacial compression and expansion performed by decreasing and increasing the drop volume at 10% of deformation amplitude (ΔA/A) and at 100 mHz of angular frequency (ω). Five oscillation cycles were followed by a time of fifty cycles without any oscillation up to the time required to reach the steady-state. Interface pressure (π, $mN{\cdot}m^{-1}$) was recorded during the test as the difference between the surface tension of aqueous solution in the absence of protein (σ^o) and the surface tension in the presence of protein (σ).

The experiments were carried out at 20 °C. All experiments were performed in duplicate, being the reproducibility of the results better than 5%.