Food Hydrocolloids xxx (2016) 1–9

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Self-assembly of a food hydrocolloid: The case of okra mucilage

S. Lousinian [b], M. Dimopoulou [a, b], C. Panayiotou [b], C. Ritzoulis [a, c, *]

[a] Department of Food Technology, ATEI of Thessaloniki, 57400, Thessaloniki, Greece
[b] Department of Chemical Engineering, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece
[c] School of Food Science & Biotechnology, Zhejiang Gongshang University, Hangzhou, Zhejiang 310018, China

ARTICLE INFO

Article history:
Received 1 September 2016
Received in revised form
16 November 2016
Accepted 18 November 2016
Available online xxx

Keywords:
Hydrogen bonding
Okra
Polysaccharides
Micro-differential scanning calorimetry
Intrinsic viscosity
Light scattering

ABSTRACT

The self-assembly of a macromolecular population of interest in food, cosmetics, and pharmaceutics, namely okra polysaccharide mucilage, has been studied by means of micro-differential scanning calorimetry (μ-DSC), static light scattering, and intrinsic viscosity measurements. The experiments, held in water–dimethylsulfoxide (DMSO) mixtures, and based on successive dilutions of DMSO solutions with water and *vice versa*, suggest the existence of history-dependent pseudo-equilibrium states, in structures where the other components of the hydrocolloid mucilage, namely proteins, should be actively partaking. A hexamer supermolecular structure, comprising of smaller supermacromolecular entities held together by hydrogen bonding, is proposed as a typical structure of okra mucilage in aqueous systems.

1. Introduction

Macromolecular assemblies of a water-soluble character play a key role in biological phenomena. As such, their importance in the mechanisms of life, including the metabolism and, more specifically, nutrition and food uptake, is crucial. The physico-chemical background of such processes is obviously related to the shape, structure, and spacial arrangement of macromolecules in the mouth, gastric, and intestinal area, where food assimilation takes place. Despite their obvious importance in life, the thermodynamic quantification of processes such as macromolecular wetting, dissolution, and structural rearrangements, of food hydrocolloids however, are far from thoroughly studied.

Understanding the thermodynamics of hydrocolloidal dispersions in food and their subsequent effects in texture and nutritional aspects should require the selection of an appropriate model hydrocolloid. A good candidate to act as a model for the macromolecular experimental thermodynamics in food is okra (*Abelmoschus esculentus* L.) extract. Also, known as lady's finger, gumbo, bamya/bamia, or bhindi, okra is a monocotyledon herbaceous plant of the Malvaceae family, with total trade estimated to over $5 billion (FAO, 2012; Fortunati et al., 2013; Georgiadis et al., 2011; Ghori, Alba, Smith, Conway, & Kontogiorgos, 2014). The pods of this plant are known to produce slimy aqueous extracts, and are used as thickeners in various parts of the world. These are naturally available, inexpensive and non-toxic biopolymers, with a substantial potential for pharmaceutical formulations (Ghori et al., 2014; Zaharuddin, Noordin, & Kadivar, 2014) or for use as bone scaffolds (Dimopoulou, Ritzoulis, Papastergiadis, & Panayiotou, 2014). The extraction, composition, rheology, and emulsifying aspects of okra mucilage have been recently reviewed by Ritzoulis (2016). Of primary interest is the thick and slimy texture of okra aqueous extracts; this is mostly due to their polysaccharide content (Dimopoulou, Ritzoulis, & Panayiotou, 2015; Dimopoulou, Tsivintzelis, Ritzoulis, & Panayiotou, 2016; Ghori et al., 2014). The principal polysaccharide components of aqueous okra polysaccharide extracts are type I partially methylated and acetylated rhamnogalacturonnans with small galactosyl residue side branches (Senghamparn, Bakx, et al., 2009; Sengkhamparn, Verhoef, Schols, Sajjaanantakul, & Voragen, 2009).

Recent work available on the role of these biomacromolecular extracts in terms of composition, rheology, emulsifying and emulsion stabilizing properties suggest a good potential as a thickening agent/rheology modifier, and as an emulsifier of acidic foods (Alba, Ritzoulis, Georgiadis, & Kontogiorgos, 2013; Alamri, Mohamed, & Hussain, 2013; Dimopoulou et al., 2014, 2015, 2016; Ghori et al.,

* Corresponding author. Department of Food Technology, ATEI of Thessaloniki, 57400, Thessaloniki, Greece.
E-mail address: critzou@food.teithe.gr (C. Ritzoulis).

http://dx.doi.org/10.1016/j.foodhyd.2016.11.024
0268-005X/

2014; Zaharuddin et al., 2014).

Considering the above, the study of the self-assembly of okra macromolecules may offer valuable insight on the structure-function relations of the biomacromolecules encountered in natural systems that are key to food texture, food intake and digestion. In this work, the molecular-level thermal properties and the hydrogen bonding interactions of the okra extract in ultra pure water and in dimethyl sulfoxide (DMSO)–water mixtures were investigated by means of micro-differential Scanning Calorimetry (μ-DSC) (Xu, Wang, Cai, & Zhang, 2010). The role of DMSO is to induce breaking of hydrogen bonds (McCormick, Callais, & Hutchinson, 1985). μ-DSC is a very sensitive technique which, when applied on unlabelled macromolecule solutions, it enables us to assess the overall structure of the molecule by observing the difference in heat flow between the sample and the reference during controlled temperature increase (Wang, Zhang, Zhang, & Ding, 2009). It provides an extremely useful approach, in order to characterise the energetics and mechanisms of temperature-induced conformational changes of macromolecules, through obtaining important information on thermodynamic and kinetic features of the thermal denaturation (Lousinian, Missopolinou, & Panayiotou, 2013). The results of this work are discussed in conjunction with intrinsic viscosity and light scattering measurements, with the objective of quantifying the contribution of hydrogen bonding to the self-assembly of okra mucilage.

2. Materials and methods

Mature okra pods, sized 5–9 cm in length, grown in the Meliki region (Imathia, Greece), where obtained from the local producers. The pods were immediately frozen and stored at −20 °C. Distilled water has been used throughout the extractions, while sodium phosphate monobasic dihydrate ($NaH_2PO_4 \cdot 2H_2O$) was purchased from Fluka (Sigma-Aldrich, >98% purity) for the phosphate buffer preparation. Milli-Q water (water purified by treatment) obtained from a Milli-Q apparatus (Millipore Corporate, MA, USA). DMSO was purchased from Fluka (Sigma-Aldrich, ≥99.9% purity) for the preparation of DMSO/water mixtures.

2.1. Okra mucilage extraction

Okra pods were freeze-dried and milled. 30 g of this dried material was subjected to extraction (600 mL at 80 °C for 30 min) with phosphate buffer (5 mM) set at pH 7.0. The solubilized extract was separated from the insoluble residue by means of filtration, and was subsequently freeze-dried. The okra solutions were prepared by dispersing 2% w/w freeze-dried okra in Milli-Q water (ultra pure water). The dispersion (100 mL) was dialysed (3500 Da Molecular weight cut-off dialysis tubing) at 20 °C for 24 h in the same solvent (2 L) and then the dialysis sample was freeze-dried. The okra fraction was dissolved, respectively, in ultra pure water, DMSO, and the DMSO/water mixtures for 24 h with stirring at room temperature to prepare the polymer solutions.

2.2. Micro-DSC measurements

Micro-DSC measurements were performed using a VP-DSC (MicroCal Inc., Northampton, MA) calorimeter, between 10 and 120 °C, at a scan rate of 1 °C min^{-1} and at okra extract concentration of 0.1 mg mL^{-1}. This study involved the study of okra purified macromolecules in ultra pure water (pH 7) and in dimethyl sulfoxide (DMSO)–ultra pure water mixtures of several ratios, with 2.5–30% v/v DMSO. DMSO was used to induce breaking of the hydrogen bonds in the system. The okra fractions were dissolved, respectively, in ultra pure water, and the DMSO/water mixtures for 24 h with stirring at room temperature to prepare the polymer solutions. In these experiments, DMSO was used in ratios of 0 (pure water) to 30% v/v. No higher DMSO concentrations were used, due to practical difficulties arising from the excessively high viscosity of the obtained solutions. Each solution has been prepared by means of dissolving an appropriate amount of okra mucilage in a DMSO–water solution of the desired composition.

Dilution experiments were performed in a following stage, in order to see if the hydrogen bonding network is formed again when the DMSO/water ratio decreases. Initially an okra solution of DMSO/water ratio 30:70 (v/v) with concentration of 0.1 mg mL^{-1} was prepared and measured by μ-DSC. Then the solution was diluted with ultra pure water, until the DMSO percentage was decreased to 17.5% v/v, 15% v/v and 5% v/v. The inverse series of experiments was also performed, starting with an okra solution of DMSO/water ratio 5:95 (v/v) with concentration 0.1 mg mL^{-1}. Then the solution was diluted with DMSO, until the DMSO percentage was increased to 7.5% v/v, 10% v/v and 15% v/v.

The heat capacity curves were evaluated using MicroCal Origin 7.0 software. The denaturation temperature (transition temperature T_m) corresponds to the temperature where the local maximum occurs in the excess heat capacity. The enthalpy of the transition (ΔH) was calculated from the area of the peak, which is related to the amount of intramolecular interactions in a macromolecule.

Concentration cycling experiments were performed as follows: A solution of 0.1 mg mL^{-1} okra extract (10% v/v DMSO) was prepared. Appropriate amounts of DMSO and water were subsequently added, aiming at a DMSO concentration cycling at 10.0%, 12.5%, 10.0%, 7.5%, and then 10.0% v/v DMSO. Cycling was kept at an intentionally narrow range, as to minimize dilution of the okra extract, and to remain close to the area of maximum endothermic activity.

2.3. Intrinsic viscosity measurements

Viscosity measurements were carried out for okra fractions with concentration of lower than 3 mg cm^{-3} in pure water, pure DMSO and DMSO/water mixtures (30:70, 17.5:82.5, 15:85) at 20.3 °C using a conventional viscometer of the Cannon–Fenske type. All the test solutions were maintained at a constant temperature within ±0.01 °C during the measurements, and the flowing time was measured to a precision of 0.1 s. The kinetic energy correction was always negligible. Huggins and Kraemer plots were used to get intrinsic viscosity [η].

$$\frac{\eta_{sp}}{c} = [\eta] + k' \cdot [\eta]^2 \cdot c \quad (1)$$

$$\frac{\ln \eta_r}{c} = [\eta] + k'' \cdot [\eta]^2 \cdot c \quad (2)$$

where k' is the Huggins coefficient, k'' the Kraemer coefficient. The linear functions were extrapolated to zero concentration to obtain the intrinsic viscosity at the intercept.

2.4. Static light scattering

The angular dependence of the intensity of scattered monochromatic light was measured by a multi-angle laser light scattering instrument (MALLS) (Brookhaven Instruments Corporation, NY) equipped with a 635 nm laser, measuring at the angles of 35, 50, 75, 90, 105, 130, and 145° at 25 °C. A dedicated software package (Brookhaven Instruments Corporation, NY), was used for data acquisition and handling. The determination of the weight average molecular weight M_w and that of the radius of gyration R_g were

Please cite this article in press as: Lousinian, S., et al., Self-assembly of a food hydrocolloid: The case of okra mucilage, *Food Hydrocolloids* (2016), http://dx.doi.org/10.1016/j.foodhyd.2016.11.024