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Relating the variation of secondary structure of gelatin at fish oil–water interface to adsorption kinetics, dynamic interfacial tension and emulsion stability

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ABSTRACT

The objectives of this study were to quantify the relationship between secondary structure of gelatin and its adsorption at the fish-oil/water interface and to quantify the implication of the adsorption on the dynamic interfacial tension (DST) and emulsion stability. The surface hydrophobicity of the gelatin solutions decreased when the pH increased from 4.0 to 6.0, while opposite tend was observed in the viscosity of the solution. The DST values decreased as the pH increased from 4.0 to 6.0, indicating that higher positive charges (measured trough zeta potential) in the gelatin solution tended to result in higher DST values. The adsorption kinetics of the gelatin solution was examined through the calculated diffusion coefficients (D_{eff}). The addition of acid promoted the random coil and β-turn structures at the expense of α-helical structure. The addition of NaOH decreased the β-turn and increased the α-helix and random coil. The decrease in the random coil and triple helix structures in the gelatin solution resulted into increased D_{eff} values. The highest diffusion coefficients, the highest emulsion stability and the lowest amount of random coil and triple helix structures were observed at pH = 4.8. The lowest amount of random coil and triple helix structures in the interfacial protein layer correlated with the highest stability of the emulsion (highest *ESI* value). The lower amount of random coil and triple helix structures allowed higher coverage of the oil–water interface by relatively highly ordered secondary structure of gelatin.

1. Introduction

Due to increased awareness of the health benefits associated with the consumption of n-3 fatty acids, the daily consumption of these health promoting fatty acids is increasing. The daily consumption of the fish oil has increased both in the liquid and in encapsulated solid form, through growing nutritional supplement and functional food markets (Kolanowski & Laufenberg, 2006). Complex coacervation has been used commercially to microencapsulate and stabilize fish oil for incorporation into a range of food products (Thies, 2007). Gelatin is the primary protein used in the application of complex coacervation to fish oil. The second, oppositely charged polymer can be gum Arabic, sodium polyphosphate, carboxymethyl cellulose or chitosan, although sodium polyphosphate appears to be used in the commercial process (de Kruif, Weinbreck, & de Vries, 2004). During complex coacervation, an important step is the formation of a (fish) oil-in-water emulsion, which is preferably stabilized or emulsified using a protein, such as gelatin. Emulsion formation occurs prior to coacervation and optimization of the emulsion process is important for obtaining stable fish-oil microcapsulates after complex coacervation. It has previously been shown that interfacial behaviour is an important aspect of both emulsion formation and stable complex coacervation (Dicharry, Arla, Sinquin, Graciaa, & Bouriat, 2006; Zhai, Wooster, Hoffmann, Lee, Augustin, & Aguilar, 2011). Here we further investigate the impact of interfacial behaviour and interfacial tension on fish-oil encapsulation using complex coacervation, with the long-term aim of enabling the use of proteins other than gelatin in this commercially useful process.

The emulsifying property of the protein is associated with its ability to decrease interfacial tension, due to adsorption of protein at water–oil interface, and the mechanical energy barrier, which provides stability against coalescence in the emulsion system (Dagorn-Scaviner, Gueguen, & Lefebvre, 1987). The lowering of interfacial tension facilitates the breakdown of oil droplets during emulsion formation. The simultaneous formation of a viscoelastic protein-rich layer between these two immiscible phases minimizes the coalescence of oil droplets during emulsion formation and storage (Mackie, Ridout, Moates, Husband, & Wilde, 2007).

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However, protein adsorption is a complex phenomenon which includes the adsorption, desorption, unfolding and aggregation, occurring simultaneously at the interface (Jung, Gunes, & Mezzenga, 2010). This adsorption process can be easily affected by various factors such as structure of protein, protein hydrophobicity and the chemical environment of the solution such as pH and ionic strength. The adsorption process can also be affected by the physical state of protein such as ageing time, concentration, and temperature, which can cause conformational change or partial denaturation of the adsorbed protein layer at the oil/water interface (de Jongh & Wierenga, 2006).

Studies on protein adsorption dynamics indicate that upon adsorption to an interface protein secondary structure can change. These changes can substantially alter the dilational rheological properties of the adsorbed protein layer, which is reflected on the changes in the interfacial properties (Pereira, Johansson, Radke, & Blanch, 2003). The structure-function properties of adsorbed protein at the interface impact the stability of a prepared emulsion (Dalgleish, 2006; Kim, Decker, & McClements, 2002). Although it is widely accepted that change in a protein's conformation impacts emulsion stability, sufficient quantitative information and detailed mechanisms are unavailable due to limitations in analytical techniques. Spectroscopic techniques are available to characterize the conformation of protein, including Fourier transform infrared spectroscopy (FTIR) (Kong & Yu, 2007), circular dichorism (CD) (Sreerama, Venyaminov, & Woody, 2000), nuclear magnetic resonance spectroscopy (NMR) (Wishart, Sykes, & Richards, 1991), atomic force microscopy (AFM) (Dauphas et al., 2007) and X-ray crystallography (Drenth & Mesters, 2007). Among these techniques, FTIR spectroscopy is widely used to estimate protein secondary structure (Smith, 2009). The amide I (C=O stretch) and amide II (in-plane NH bending) are characteristic vibrational bands in protein IR spectra, and specific bands are indicative of α-helix, β-sheet, β-turns and random coils (Kong & Yu, 2007). The quantitative analysis of protein's secondary structure is commonly undertaken. Four commonly used methods to analysis protein FTIR spectra include Fourier self deconvolution (FSD)-curve fitting(Kauppinen, Moffatt, Mantsch, & Cameron, 1981), second derivative analysis (Dong, Huang, & Caughey, 1990), partial least-squares analysis (Lee, Haris, Chapman, & Mitchell, 1990) and data basis analysis (Sarver & Krueger, 1991). Among these analysis methods, FSD-curve fitting and second derivative analyses are the two most widely used methods. For the second derivative method, peak positions correspond to different secondary structures and curve fitting enables quantitation of each structural type (Kalnin, Baikalov, & Venyaminov, 1990). Therefore, by examining the conformation of gelatin and the change in its secondary structure under various processing conditions, its ability to stabilizing the oil–water interface can be quantified.

The objective of the current study was to investigate the effect of protein conformation, hydrophobicity, pH environmental on the interfacial behaviour of gelatin at the water–fish oil interface. Furthermore, the changes in the gelatin conformation were also investigated in order to explain the interfacial behaviour of gelatin at fish oil–water interface.

2. Materials and methods

2.1. Materials

Gelatin from porcine skin (type A, 300 bloom, M_w average 50,000–100,000) was purchased from Sigma–Aldrich. The fish-oil was purchased from Sigma–Aldrich and was stored in a desiccator maintained at 4 °C to prevent oxidisation. To avoid the oxidation of the fish oil during experiments, it was kept in 5 ml glass vial with screw-tight lid on. Both the oil and the gelatin were used as received.

2.2. Methods

2.2.1. Preparation of gelatin solution

Ultrapure water (ZFMQ23004, MilliPore, NSW, Australia) was used in all solutions. The gelatin solutions (1 wt%) were prepared by adding 1 g of gelatin powder in 49 ml of cold water under agitation speed of 600 rpm at 40 °C. Clear gelatin solution was obtained after stirring for 30 min at 40 °C.

2.2.2. Measurement of interfacial properties and modeling of adsorption kinetics

2.2.2.1. Measurement of dynamic interfacial tension. The principle of the drop profile analysis, used in this study, is based on determination of the coordinates of a liquid drop from a video image and the comparison of these coordinates with theoretical profiles calculated from the Gauss Laplace equation (Rotenberg, Boruvka, & Neumann, 1983). The dynamic interfacial properties of gelatin solution at different pH and concentrations were measured with a drop profile tensiometer PAT-1 using the pendent drop method (SINTERFACE Technologist, Germany). The measurement chamber was directly connected to a syringe with the aid of a plunger and a screw thread. A drop of fish-oil was formed at the tip of a stainless steel capillary needle immersed into a curette filled with the gelatin solution. The drop volume was kept constant at 10 mm^3, except in the case where the effect of the size of an oil droplet was investigated. The temperature of the test chamber was kept constant at 40 ± 0.5 °C using a temperature controlled circulating water bath (Cool Tech 320, Germany). The tensiometer was calibrated at the air–water (69.6 mN/m) interface 40 ± 0.5 °C. The pH of the gelatin solution was adjusted (from 4.0 to 6.0 at 0.4 interval) and monitored using a pH meter (LC80A, TPS, Brisbane, Australia) by adding 5%(w/w) acetic acid or 2% (w/w) NaOH before the DST measurement. The pH values of the solution were determined by using a pH meter at the beginning and at the end of the experiments. The ΔpH values ($\Delta pH = pH_{begin} - pH_{end}$) were kept less than 0.5 for each test in order to make the DST values representative of the experimental pH values.

2.2.2.2. Modeling of the adsorption kinetics of gelatin. The adsorption kinetics of gelatin solution under various condition was simulated using the model proposed by Miller et al., based on the simultaneous solution of the Ward–Tordai equation [Eq. (4)] and a set of protein-state related equations [Eqs. (1)–(3)], corresponding to the equilibrium state of the adsorbed layer (Miller, Fainerman, Aksenenko, Leser, & Michel, 2004). This model was successfully applied previously in protein kinetics studies and the predictions essentially agreed with the experimental data. Further details of the derivation of the governing equations and the solution procedure are available in the cited publications (Fainerman, Lucassen-Reynders, & Miller, 2003; Kotsmar et al., 2008; Reis et al., 2008). A basic assumption of the model is that the protein molecules exist in several states with various molar areas varying from a minimum value (ω_{min}, m^2/mol) at highest surface coverage and maximum value (ω_{max}, m^2/mol) at lowest surface coverage. Also, ω_0 (m^2/mol) in Eq. (1) denotes the increments of the molar areas of two "neighboring" conformations. ω_0 can be chosen to be either equal to the molar area of the solvent, or the area occupied by one segment of the adsorbed protein molecule. The set of equations described by the theory are represented by Eqs. (1)–(4), given below; where Eqs. (1)–(3) describe the evolution of protein adsorption states during the formation of an adsorption layer and Eq. (4) relates the dynamic surface adsorption $\Gamma(t)$ (mg/m^2) with the