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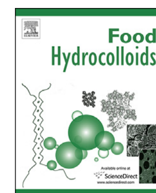

Colloidal stability and dilatational rheology at the air–water interface of peptides derived from thermal-acidic treated wheat gluten

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Defatted wheat gluten proteins were subjected to a thermal process under mildly acidic conditions using 50–250 mM HCl. The degree of deamidation and hydrolysis reached in gluten proteins produced variable water-soluble fractions at neutral pH. When pH shifted from alkaline to acidic ranges, these fractions, in buffered aqueous media, developed different zeta potentials and different association/aggregation states. Interfacial rheological properties of water-soluble peptides were characterized by dilatational rheology at the air–water interface. A pendant drop tensiometer was used to produce harmonic perturbations to obtain the elastic component of the interfacial viscoelasticity modulus. Gluten proteins with the lowest degree of hydrolysis and deamidation, produced peptides with the highest interfacial elastic moduli of adsorbed films at the air–water interface. The mildly acidic treatment produced water-soluble peptides at neutral pH with different molecular weight profiles; a peptide fraction with molecular weight lower than 10 kDa increased with the increasing HCl concentration.

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1. Introduction

Wheat gluten proteins are a renewable source of edible and biodegradable surfactants (Hesser, 1989). However, their insolubility in water limits their use as emulsifiers or foaming agents, for example (Day, Augustin, Batey, & Wrigley, 2006). Their high molecular weight, lack of ionizable amino acid residues such as glutamic acid, high concentration of nonpolar amino acid residues such as proline, and the formation of hydrogen bonding between the side chains of the polar non-ionizable amino acids such as glutamine, contribute to their insolubility, in aqueous solutions at neutral pH (Krull & Inglett, 1971; Krull & Wall, 1969; Liao, Liu, et al., 2010; Wu, Nakai, & Powrie, 1976).

Previous studies have successfully obtained water-soluble surface-active compounds from wheat gluten proteins via chemical modification. The high content of glutamine residues in gluten proteins (about one-third of the total amino acid content) (Krull & Wall, 1969) makes thermal, mildly acidic treatments a convenient way to modify these proteins. Such treatments cause the deamidation of amide groups (RCO-NH_2) from the side chains of asparagine and glutamine residues, and the hydrolysis of peptide bonds (RCO-NHR′) from wheat gluten native proteins (Berti et al., 2007; Liao, Liu, et al., 2010). Deamidation, catalyzed by acids (nucleophiles), is a hydrolytic reaction that requires a water molecule. A general acid HA catalyzes a reaction to protonate the amide group of the amino acid side chain. As a result, the conjugate base A^- can attack the carbonyl carbon of the amide group or can activate some other nucleophile to attack the amide (e.g., activate the OH^- by subtracting a proton from a water molecule). The reaction yields the corresponding carboxylic acid (R–COOH) in the amino acid residue (Wright, 1991). This produces proteins or peptides with a higher number of carboxylic acid moieties, which contributes to their solubility in water at neutral pH, because of the negative net charge development by the R–COOH groups.

Among the acids that have been used for the deamidation of wheat gluten proteins are hydrochloric acid (Friedli, 1996; Liao, Qiu, et al., 2010; Matsudomi, Kato, & Kobayashi, 1982; Mimouni, Raymod, Merle-Desnoyers, Azanza, & Ducastaing, 1994; Wu et al., 1976), citric acid, succinic acid (Liao, Liu, et al., 2010), and acetic acid (Friedli, 1996; Liao, Qiu, et al., 2010; Wu et al., 1976). Changes reported in the chemistry of wheat gluten proteins have included modifications on both the primary and secondary structural levels, in the form of several degrees of deamidation and hydrolysis. No changes in the presence of thiol groups or disulfide moieties have been observed. (Liao, Liu, et al., 2010; Liao, Qiu, et al., 2010; Matsudomi et al., 1982; Mimouni et al., 1994; Wu et al., 1976).

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The chemical changes mentioned above, have been related to different colloidal and surface properties, and consequently to the emulsifying and foaming functionality of deamidated wheat gluten proteins. Due to their nature as polyelectrolytes, effects on their functionality (and colloidal stability) as emulsifiers caused by pH and the presence of cations (K^+, Na^+ and Ca^{2+}), have also been studied (Day, Xu, Lundin, & Wooster, 2009). Their ability to decrease the air–water interface tension, i.e., their ability to be adsorbed at the air–water interface, has been related to the degree of deamidation and hydrolysis (Matsudomi et al., 1982).

In addition to their tensoactive properties, the functionality of proteins as emulsifiers or foaming agents has been directly related to the mechanical properties of the adsorbed protein film at air–water or oil–water interfaces (Benjamins & Lucassen-Reynders, 2009; Dickinson, 1999; Murray, 2011). Colloidal systems, such as emulsions and foams, are protected against several processes of destabilization, e.g., coalescence in emulsions, by the protein films adsorbed at the interface (Dickinson, 1999; Murray, 2011).

Protein films provide fluid interfaces with mechanical properties like elasticity and viscosity (viscoelastic properties) that enable interfaces to resist stresses from the adjoining flowing fluids (Benjamins & Lucassen-Reynders, 2009). Interfacial viscoelasticity is a derivation of the surface elasticity, E, defined by the Gibb's equation (Eqn. (1)):

$$E = \frac{d\sigma}{dInA} \qquad (1)$$

where σ represents the interfacial tension, and A represents the total interfacial area. Pure surface elasticity can occur when an interface undergoes a change to its surface area, and instantly adjusts the surface tension towards an equilibrium value in response. On the other hand, interfacial viscoelasticity appears when relaxation processes that take place in the interface affect either the σ, or the surface concentration of the surfactant (Γ). In this case, E, represents the interfacial viscoelasticity, whereby moduli for both interfacial elasticity and interfacial viscosity contributed to the total value. Surface viscoelasticity can be measured by surface compression/dilatation, which measures the response to changes in area, when shape of the surface element remains constant.

Among several instruments available to measure fluid interfacial rheological properties, automatic pendant drop tensiometers permit the measurement of dilatational rheology of protein-adsorbed films. This type of tensiometer can perform dilatational rheological tests through harmonic expansion/compression cycles of A (Eqn. (2)), which implies the interfacial tension response (Eqn. (3)) to changes in area, at a constant shape of a surface element; the interfacial tension oscillates at the same frequency as area, however, with a phase shift (phase angle) δ (Rühs, Affolter, Windhab, & Fischer, 2013).

$$A = A_0 + \Delta A(\sin \omega t) \qquad (2)$$

$$\sigma = \sigma_0 + \Delta\sigma(\sin \omega t + \delta) \qquad (3)$$

where A_0 is the initial or reference interfacial area, ΔA the amplitude of the area oscillations, ω the frequency imposed, σ_0 the equilibrium reference of interfacial tension, $\Delta\sigma$ the amplitude of the interfacial tension oscillations, and t represents time. In such case, E, is a complex number, with a real part E_d (the storage modulus) equal to the interfacial elasticity and the imaginary part E_η (the loss modulus), as given by the product of the interfacial viscosity η_d, and ω of the area variations (Eqn. (4)) (Benjamins & Lucassen-Reynders, 2009).

$$E = E_d + E_\eta = E_d + i\omega\eta_d \qquad (4)$$

The aim of this work was to investigate the relationship between interfacial dilatational properties, at the air–water interface of adsorbed water-soluble peptides, derived from thermal mildly acidic-treated wheat gluten. In addition, some of the colloidal and molecular characteristics of such peptides in neutral aqueous solution were investigated.

2. Materials and methods

2.1. Standards and reagents

Vital wheat gluten flour (75% of protein, AOAC method 960.52; 1.2% fat, AOAC method 920.85) was provided by Gluten y Almidones Industriales (Mexico City, México). DC Protein Assay, broad-range protein standards, and other electrophoresis reagents were purchased from Bio-Rad (Laboratories, Inc., Hercules, CA, USA). Sodium dodecyl sulfate and trichloroacetic acid (ACS reagent) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). All other chemicals were analytical grade from Fermont (Monterrey, México). Spectra/Por dialysis membrane with a molecular weight cut off of 6000–8000 was purchased from Spectrum Laboratories Inc. (Rancho Domínguez, CA, USA).

2.2. Sample preparation

Gluten flour was defatted overnight in a soxhlet apparatus using chloroform:methanol (2:1, v/v) solution. After defatting, the fat content was 0.45% (AOAC method 920.85). Remaining solvents were evaporated from defatted flour at room temperature. Next, 10% defatted wheat gluten flour suspensions were prepared in 50–250 mM HCl. First, the suspensions were mixed with a vortex stirrer, and after a hydration period of 2 h at room temperature, they were subjected to heating in an autoclave at 121 °C/103.4 kPa for 10 min. After heating, samples were placed in an ice water bath for 20 min to stop the reaction, followed by neutralization with 500 mM NaOH. Once suspensions were neutralized, they were dialyzed against sodium-free purified water at 4 °C for 12 h, and then dialyzed against a 0.01% sodium azide solution at 4 °C for 12 h.

After dialysis, neutralized wheat gluten suspensions were centrifuged at 1500 × g for 10 min at room temperature, the pellet was discarded and the supernatants, containing the soluble peptide fractions at pH 7, were conserved and used to obtain soluble peptide stock solutions at the same neutral pH. Protein concentration of the supernatants was determined in duplicate with the DC Protein Assay at 750 nm according to manufacturer's specifications, using a DU-64 Spectrophotometer (Beckman Coulter Inc., Fullerton, CA, USA). The concentrations of all supernatants were adjusted with a 0.01% sodium azide solution to a protein concentration of 1%; these stock solutions were conserved in sterile glass containers at 4 °C until use, and used without any further dilution or concentration directly to the study of the interface properties. Water-soluble peptides are referred to in the text as 50 mM, 100 mM, 150 mM, 200 mM, and 250 mM peptides, which correspond to those obtained using 50 mM, 100 mM, 150 mM, 200 mM, and 250 mM HCl, respectively.

Stock solutions were used for zeta potential, size distribution and turbidimetric measurements, prepared as follows: an aliquot of 50 μL of stock solution was diluted in 1 mL of the respective buffer solution of phosphates at pH 6, 7 and 8, and acetates at pH 4 and 5 to reach a final sample concentration of 0.05% (w/v).

When the pH was lowered to acidic values (4 and 5), water-soluble peptides obtained initially at pH 7 produced the

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