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Understanding the stability mechanisms of lentil legumin-like protein and polysaccharide foams

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ABSTRACT

Foaming properties of lentil legumin-like protein were investigated in the presence of guar gum, xanthan gum and pectin at different environmental pH conditions (3.0, 5.0, and 7.0). The protein foaming capacity was not significantly impacted by adding polysaccharides, whereas foam stability was greatly enhanced at pH 3.0 and 5.0, leading to formation of long-life foams in most samples with the highest mean life value of 275 min at pH 5.0 in the presence of pectin. Investigation of the stability mechanisms revealed that at pH 3.0, the presence of the coacervates stabilized the foams against collapse due to the formation of an electrostatically cross-linked gel-like interfacial network. At pH 5.0, aggregates were formed that adsorbed to the interface to form stiff and thick interfacial network, avoiding foam coarsening. Aggregates also plugged the junctions of the Plateau borders, slowing down the drainage by a jamming effect, and dramatically increased apparent viscosity of the foams, thus favoring the immobilization of the lamellar water surrounding the gas bubbles. The thermodynamic incompatibility at pH 7.0 resulted in a phase separation of protein and polysaccharide in the interfacial protein membrane. This induced a disruption of the protein layer around the bubbles making it weaker and easier to break, leading to reduced foaming stability. The findings revealed that guar, xanthan, and pectin can improve the stability of lentil legumin-like protein foams at mild acidic pH, creating long-life foams, which would be particularly useful in the food industry where aerated structures must be preserved for a long period of time before solidifying or gelling.

1. Introduction

A foam can decay smoothly or collapse very fast. Foam mean life value is considered as an index of foam stability, which is the inverse of the decay constant of a foam system (Hackbarth, 2006). Long-life foams, which normally show mean life value of 50 min or higher (Piazza, Gigli, & Bulbarello, 2008) are particularly useful in the food industry when products must be processed for long periods and it is important to keep the aerated structure before solidifying or gelling (e.g. mousse and ice cream). Formation and stabilization of foams are to a large extent determined by the properties of the compounds they contain at the air/water interface. The presence of proteins empowers these properties due to a combination of their surface activity, and electrostatic and steric mechanisms (Foegeding, Luck, & Davis, 2006).

Lentil is a leguminous plant low in fat and high in dietary fibre and other nutrients, such as protein, dietary fiber, vitamins and minerals (Roy, Boye, & Simpson, 2010; Thavarajah, Thavarajah, & Sarker, 2009) and its consumption has been associated with several health benefits (Barbana & Boye, 2011; Roy et al., 2010). Lentil contains 20.6–31.4% protein with legumin-like protein (~50%) as the major globulin fraction (Urbano, Porres, Frias, & Vidal-Valverde, 2007). Generally, it is accepted that legumin is a hexamer with a molecular weight (M_w) of about 320–380 kDa, which consists of six polypeptide pairs that interact non-covalently. Each of these polypeptide pairs is comprised of an acidic subunit of about 40 kDa and a basic subunit of about 20 kDa, linked by a single disulfide bond (Urbano et al., 2007). Lentil legumin-like protein has an isoelectric point (*pI*) around 4.6 with balanced hydrophilic (~38%) and hydrophobic (~40%) residues. It also possesses both high

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surface hydrophobicity and solubility when pH is deviated from its *pI* (Jarpa-Parra et al., 2015). Thus, lentil legumin-like protein demonstrated excellent foaming capacity and stability at pH 5.0 and 7.0, respectively (Jarpa-Parra et al., 2015).

Protein foam properties can be improved by the addition of polysaccharides, and the foam stability depends on the interactions between these two kinds of biopolymers and the ability of their mixture to retard gravity-induced drainage by controlling the rheology and network structure of the continuous phase (Narchi, Vial, & Djelveh, 2009; Rodríguez-Patino & Pilosof, 2011). Complex coacervation, miscibility, and segregation are the possible phenomena that arise in aqueous solutions of protein and polysaccharide mixtures (Narchi et al., 2009). Using anionic polysaccharide as an example, if the pH of the aqueous medium is reduced to below the isoelectric point (pI) of the protein, complex coacervation occurs as a result of net electrostatic attraction between the oppositely charged biopolymers coupled with phase separation, one rich in the complexed biopolymers and the other phase depleted in both. Above the isoelectric point, because of the repulsive electrostatic interactions and different affinities towards the solvent, thermodynamic incompatibility of protein and polysaccharide occurs. Above a critical concentration and/or at high ionic strength, protein and polysaccharide segregate into different phases. However, if their concentration is sufficiently low, they could co-exist in a single phase (miscibility) in which they mutually exclude one another (Rodríguez-Patino & Pilosof, 2011).

The impact of protein-polysaccharide interactions on the dynamics of protein adsorption at the interface and the effect on foam capacity has been studied previously (Miquelim, Lannes, & Mezzenga, 2010; Sadahira et al., 2015; Van den Berg, Jara, & Pilosof, 2015). Most research studied the influence of the protein-polysaccharide interactions just at one pH value, normally below pI. In one of the most recent studies, Ruíz-Henestrosa, Carrera-Sánchez, and Patino. (2008) analyzed the effect of sucrose on dynamic surface pressure, surface dilatational properties and foam characteristics of soy globulins (7S and 11S) at pH 7.0 and 5.0. As pH plays an important role to determine protein polysaccharide interactions, its impact on surface properties of the complex systems should be investigated in a broad range of pH conditions applied for food applications. In addition, a fundamental understanding of the impact of polysaccharide addition on the molecular structure of lentil protein at the foam interface, subsequently the foaming properties of the complex system has never been reported.

The purpose of the present study was to investigate how the presence of different polysaccharides may impact the lentil legumin-like protein capacity to stabilize foams. It is hypothesized that a synergistic effect could be achieved by modulating protein-polysaccharide interactions for improved foam stability. The polysaccharides selected for this study are guar gum, xanthan gum, and pectin, representing non-ionic and anionic polysaccharides with different molecular weights. These are widely used in food applications as thickening or gelling agents and stabilizers because of their water-binding properties. Guar gum is a non-ionic galactomannan with *Mw* of 10^6 Da. Xanthan gum is a stiff high molecular weight anionic polysaccharide with *Mw* of 2×10^6 to 5×10^7 Da that can form highly viscous solutions. Pectin is a commonly used anionic polysaccharide with *Mw* of 2×10^4 to 4×10^5 Da. The two major commercially available pectins have typical DE values of 70% and 35%, corresponding to high methoxyl (HM) and low methoxyl (LM) pectin, respectively (De Jong & Van de Velde, 2007). The impact of polysaccharides of different molecular structures (i.e. molecular weight and surface charge) on protein surface properties (surface tension, dilatational and shear rheology), and subsequently foaming functionality was systematically investigated at different environmental pH values.

2. Materials and methods

2.1. Raw materials

Lentil legumin-like protein extract (83% w/w) was obtained by rate-zonal centrifugation using a sucrose lineal density gradient as described in our previous work (Jarpa-Parra et al., 2015). The remaining part consisted of 5.0% of water, 2.4% of ash, 1.6 of lipids, and 8.0% of carbohydrates (mainly starch). Guar and xanthan gums, Rhodamine B, and Calcofluor White (Fluorescent brightener 28) were obtained from Sigma-Aldrich Canada Co. (Oakville, Ontario, ON, Canada). The LM Pectin was provided by MP Biomedicals, LLC (Solon, OH, USA). All other chemicals were reagent-grade.

2.2. Preparation of the protein-polysaccharide mixtures

Pectin, guar or xanthan gum (1 mg/mL) solution was mixed with the lentil legumin-like protein (10 mg/mL) solution by magnetic stirring. Mixtures were prepared at three different pH levels (3.0, 5.0, and 7.0) adjusted using 0.1 M HCl or NaOH.

2.3. Electrophoretic mobility

The electrophoretic mobility of the protein-polysaccharide mixtures within the pH range of 2.0–11.0 (adjusted using 0.1 M HCl or NaOH) at 22 °C was measured by laser Doppler velocimetry using a Zetasizer NanoS (model ZEN1600, Malvern Instruments Ltd., Malvern, UK) as described previously (Jarpa-Parra et al., 2015).

2.4. Foam properties

Foaming capacity was determined as in our previous study (Jarpa-Parra et al., 2014). Briefly, 30 mL of protein-polysaccharide mixtures were mixed for 2 min with a homogenizer (PowerGen 1000, Fisher Scientific, Fairlawn, NJ, USA) at speed six. The foaming capacity (FC) was calculated as: $FC\ (\%) = (V_{f\,1} - V_{f\,0})/V_{f\,0} \times 100$, where $V_{f\,0}$ and $V_{f\,1}$ represent the volumes of the protein-polysaccharide mixture and the formed foams after homogenization, respectively.

As an index of foam stability (*FS*), a "mean life" τ value was determined according to Equation (1) (Hackbarth, 2006). The larger the τ value, the longer is the foam stand time.

$$H(t) = H(0)e^{-\lambda t} \tag{1}$$

where *H(0)* is the initial foam height at time t = 0, and λ is the decay constant, which is a measure of foam decay. This exponential relationship can be converted to a linear equation by taking the natural logarithm of the foam height, $\ln(H(t))$, vs time, where the slope value corresponds to $-\lambda$. τ is the inverse of a decay constant of a foam system.

$$ln[H(t)] = ln[H(0)] - \lambda t \tag{2}$$

$$\frac{d[ln(H)]}{dt} = -\lambda = \frac{1}{\tau} \tag{3}$$

Foam drainage was also calculated by measuring the volume of the drained liquid from the foams in a graduated cylinder over a period of 1 h and expressed as the liquid fraction (ε) in the foam ((volume of liquid − volume of drained liquid)/volume of foam) (Salonen, In, Emile, & Saint-James, 2010).