Food Hydrocolloids 88 (2019) 119–126

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Effect of fish gelatine-sodium alginate interactions on foam formation and stability

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ARTICLE INFO

Keywords:
Fish gelatine
Alginate
Foams
Surface tension
Stability

ABSTRACT

The effect of fish gelatine (FG) – alginate (AL) interactions on the formation and stability of foams was investigated by examining relationships between surface, bulk, and foaming properties of aqueous mixtures of FG and AL at 25 °C under different values of pH and FG:AL ratio. Replacing a portion of FG with AL (FG:AL ratio = 80:20, 50:50, and 20:80) at pH 5.0 or 7.0 increased the air-liquid surface tension, negative electrophoretic mobility, bulk viscosity, and particle size of FG – AL mixtures. At pH 3.5 (below the isoelectric point of FG), the AL replacement increased the particle size more dramatically; however, it suppressed trends of increasing negative electrophoretic mobility and bulk viscosity, and even reduced the surface tension, due to stronger electrostatic attractions between oppositely charged FG and AL molecules and the resulting formation of more charge-neutralised FG – AL complexes. Foaming ability became stronger as the surface tension decreased, the negative electrophoretic mobility approached to zero (more charge-neutralised), and the bulk viscosity decreased; however, it was not closely correlated with particle size. FG – AL mixtures had a weaker foaming ability than solutions prepared only with FG or whey protein concentrate; however, these mixtures exhibited much higher foam stability during storage at 25 °C. FG – AL mixtures prepared at pH 3.5 and a FG:AL ratio of 80:20 showed the best foaming ability and foam stability.

1. Introduction

Foams are a type of dispersed systems that consist of a gaseous phase dispersed in a continuous liquid or a solid phase. They are the basic component of a variety of food products including whipped cream, desserts, smoothies, mousses, marshmallow, meringues, and ice cream. Proteins such as egg white, soy, or whey protein are the most widely used macromolecular foaming agents in the food industry (Liszka-Skoczylas, Ptaszek, & Żmudziński, 2014; Miquelim, Lannes, & Mezzenga, 2010). Polysaccharides (such as xanthan gum, guar gum, and κ-carrageenan) are often used to improve the stability of protein-based foams. These materials improve foam stability by different mechanisms, including thickening effects, and interactions (attractive or repulsive) between proteins and polysaccharides (Dickinson, 2003; Liszka-Skoczylas et al., 2014; Miquelim et al., 2010; Narchi, Vial, & Djelveh, 2009).

Proteins may undergo either attractive or repulsive interactions with polysaccharides, depending on conditions within aqueous medium conditions (Razzak, Kim, & Chung, 2016; Yang, Anvari, Pan, & Chung, 2012). Attractive interactions are driven mostly by electrostatic interactions between positively charged proteins and negatively charged polysaccharides. These interactions induce the formation of either soluble or insoluble biopolymer complexes. Repulsive interactions are dominant when proteins and polysaccharides are uncharged or similarly charged, resulting in thermodynamic incompatibility between the two biopolymers (Harnsilawat, Pongsawatmanit, & McClements, 2006). These interactions form a two-phase system consisting of protein-rich and polysaccharide-rich phases at high biopolymer concentrations, but at dilute concentrations, the two biopolymers are cosoluble in a single phase. Previously, Miquelim et al. (2010) showed that the stability of egg albumin-based foam was enhanced by the addition of κ-carrageenan at a pH below the protein's isoelectric point due to attractive

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https://doi.org/10.1016/j.foodhyd.2018.09.041
Received 17 February 2018; Received in revised form 24 September 2018; Accepted 26 September 2018
Available online 27 September 2018
0268-005X/

protein-polysaccharide interactions. Perez, Sánchez, Patino, Rubiolo, and Santiago (2010) reported that surface and viscoelastic properties of β-lactoglobulin or whey protein concentrate at the air-water interface were improved by the addition of xanthan gum; they attributed their results to repulsive protein-polysaccharide interactions that could induce thermodynamic incompatibility and segregative separation of the biopolymers.

Fish gelatine (FG) has been proposed as an alternative to mammalian gelatines as it dispels consumer concerns regarding both cultural/religious dietary restrictions and contamination with bovine spongiform encephalopathy, and can be easily obtained from by-products of the fishery industry (Karim & Bhat, 2009; Yang et al., 2012). FG has lower contents of proline and hydroxyproline (about 17–25% of total amino acids) than mammalian gelatines (about 30% of total amino acids) (Karim & Bhat, 2009). The hydroxyl group of proline and hydroxyproline can form hydrogen bonding with water molecules, and therefore, FG shows different physicochemical and functional properties, including weaker gelling properties, compared to mammalian gelatines due to less hydrogen bonding (Karim & Bhat, 2009). Previous studies showed that FG obtained from the skin of different fish species – including sole, squid, cuttlefish, unicorn leatherjacket, grey triggerfish, and channel catfish – exhibited comparable foaming properties to mammalian gelatines (Aewsiri, Benjakul, Visessanguan, Wierenga, & Gruppen, 2011; Ahmad & Benjakul, 2011; Duan, Zhang, Liu, Cui, & Regenstein, 2018; Giménez, Alemán, Montero, & Gómez-Guillén, 2009; Jellouli et al., 2011; Nagarajan, Benjakul, Prodpran, Songtipya, & Kishimura, 2012). However, the foaming properties of FG in the presence of polysaccharides have not been previously reported.

Alginate (AL) is a linear anionic polysaccharide that consists of 1,4-linked-α-L-guluronic acid and β-D-mannuronic acid groups. It is found mainly in brown seaweeds, and is widely used as a thickener, stabilizer, or gelling agent in foods, pharmaceuticals, and cosmetics preparations (Razzak et al., 2016). Razzak et al. (2016) demonstrated that AL interacts electrostatically with FG in aqueous media to form either solid-state insoluble complexes (i.e. precipitates) or soluble complexes depending on pH, the FG-to-AL weight ratio (FG:AL ratio), total biopolymer concentration, and ionic strength.

The objective of this study was to investigate the effect of FG − AL interactions on the formation and stability of foams. This was achieved by examining the relationship between the surface, bulk, and foaming properties of aqueous mixtures of FG and AL – including surface tension, electrophoretic mobility, viscosity, particle size, foam ratio (FR), and bubble size – under different values of pH and FG:AL ratio, which are known primary factors influencing interactions between FG and AL. For comparison purpose, the properties of whey protein concentrate (WPC), often used as a commercial foaming agent in food industry (Liszka-Skoczylas et al., 2014), were also examined.

2. Materials and methods

2.1. Materials

FG derived from the skin of cold-water fish (including cod, Pollock, and haddock; 80.2% protein, 8.7% carbohydrates, 0% lipid, 11.0% moisture, and 0.1% ash) and AL from brown algae (extra pure sodium salt; mannuronic acid to guluronic acid ratio = ~0.8; 3.4% protein, 60.8% carbohydrate, 0.5% lipid, 10.9% moisture, and 24.4% ash) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) and Kanto Chemical Co., Inc. (Tokyo, Japan), respectively. Commercial whey protein concentrate (WPC; 65.7% protein, 24.5% carbohydrate, 1.4% lipid, 5.5% moisture, and 2.6% ash) was obtained from Hilmar Cheese Company, Inc. (Hilmar, CA, USA). The proximate compositions of the biopolymers were determined using Kjeldahl method for protein, phenol-sulfuric acid method for carbohydrate, Soxhlet method for lipid, and Karl-Fisher method for moisture. The weight average molar mass of FG (56 kDa), AL (233.4 kDa), and WPC (20.9 kDa) were measured by high performance size exclusion chromatography coupled to multiangle laser light scattering and refractive index detection system (HPSEC-MALLS-RI) according to the method of Yang, Chung, and You (2008) with slight modifications. Sodium azide (NaN_3) was obtained from Daehung Chemicals & Metals (Siheung, Korea).

2.2. Preparation of biopolymer mixtures

Aqueous solutions of FG and AL were prepared separately in a citrate-phosphate buffer at pH 3.5, 5.0, or 7.0 with 0.02% (w/v) of sodium azide as a preservative. These solutions were placed in a water bath and shaken at 100 rpm for 24 h at either 40 °C (FG) or 80 °C (AL) to ensure complete dissolution of the biopolymers. FG and AL solutions were then cooled to 25 °C and mixed to prepare several 50 mL FG − AL mixtures with a total biopolymer concentration of 0.5% (w/v) at different values of FG-to-AL weight ratio (FG:AL rato = 100:0, 80:20, 50:50, 20:80, and 0:100). The mixtures were stirred for 90 min to ensure sufficient biopolymer interactions, and stored at 25 °C for 24 h to reach equilibrium before experiments were performed. Aqueous solutions of WPC (0.5%, w/v) were also prepared separately at pH 3.5, 5.0, or 7.0 using the same method used to prepare FG solutions.

2.3. Measurement of biopolymer mixture properties

The surface tension (γ, mN/m) of the biopolymer solutions and mixtures at the air-liquid interface was measured at 25 °C via the Wilhelmy plate method using an automated force tensiometer (K100; Krüss GmbH, Hamburg, Germany). The electrophoretic mobility (μ, mm cm/V s) of biopolymers in the biopolymer solutions and mixtures was determined at 25 °C by laser Doppler electrophoresis combined with phase analysis light scattering (PALS) (Zetasizer Nano ZS; Malvern Instrument, Worcestershire, UK). The apparent viscosity (η, cP) of the biopolymer solutions and mixtures was determined using a rotational viscometer (LVDV–III Ultra; Brookfield Engineering Laboratories Inc., Middleboro, MA, USA) equipped with SC4-18 spindle at 25 °C at a constant shear rate of 79.2 s^{-1} (60 rpm).

The volume-weighted mean diameter ($d_{4,3}$, nm) of biopolymers in the biopolymer solutions and mixtures was determined using dynamic light scattering. The fluctuation in scattered light intensity by the Brownian motion of biopolymers was detected at 25 °C using the Zetasizer. The intensity fluctuation was converted to a correlation coefficient, an expression for the time dependence of the fluctuation in scattered light intensity, and then to diffusion coefficient (D, m^2/s), followed by the calculation of hydrodynamic diameter (d_H, nm) using the Stoke-Einstein equation:

$$d_H(\times 10^6) = \frac{kT}{3\pi\eta D} \quad (1)$$

where k is the Boltzmann's constant (1.38×10^{-23} J/K), T is the absolute temperature (K), and η is the apparent viscosity (cP). The intensity-weighted distribution of d_H was generated and transformed to the volume-weighted distribution of d_H using the Mie theory to obtain the $d_{4,3}$ (Bhattacharjee, 2016; Stetefeld, McKenna, & Patel, 2016). Polydispersity index (PDI) was also obtained from the correlation coefficient using a cumulants analysis method. All data handling was carried out using the Zetasizer Nano software (Malvern Instrument, Worcestershire, UK).

2.4. Preparation of foams

Aqueous solutions of FG, AL, and WPC and aqueous FG − AL mixtures were prepared at 25 °C as described in Section 2.2. A volume of 50 mL of each biopolymer solution or mixture was transferred to a 100 mL cylinder and homogenized at 16,000 rpm for 2 min using an Ultra-Turrax T25 digital homogenizer (IKA, Staufen, Germany) to incorporate air.

2.5. Measurements of foam properties

The foam ratio (FR) was measured as the ratio between the volume of foams created in the cylinder and the initial volume of the biopolymer solution or mixture (50 mL). The foam volume was measured as a function of time (0, 6, 12, 18 and 24 h after foam formation) at 25 °C. The foam bubble diameter was measured as a function of time (0, 6, and 12 h after foam formation) at 25 °C using a stereomicroscope (Leica MZ12.5 with DFC290 digital camera; Leica Microsystems Inc., Wetzlar, Germany) at a magnification of 160 ×. At least 100 bubbles were measured to calculate average values.

2.6. Statistical analysis

All experiments were performed at least in triplicate, and all measurements were repeated at least three times. Data are expressed as mean ± standard deviation. Pearson correlation analysis was conducted between FR and the four biopolymer mixture properties (γ, μ, η, and $d_{4,3}$) using IBM SPSS Statistics 20.0 (SPSS Inc., Chicago, IL, USA), where all p-values were two-tailed with a significant level of 0.05.

3. Results and discussion

3.1. Properties of biopolymer mixtures and solutions

3.1.1. Surface tension

The surface tension (γ) of FG solutions (FG:AL ratio = 100:0) was measured between 43.9 and 48.9 mN/m, depending on the pH (Fig. 1). The lowest value of γ was obtained at pH 5.0, while γ values at pH 3.5 and 7.0 were similar. We previously reported that the isoelectric point of FG was 5.4 at 25 °C (Razzak et al., 2016). Thus, intermolecular electrostatic repulsion would be weaker at pH 5.0 than at pH 3.5 or 7.0. This weaker repulsion could induce higher packing of FG molecules at the air-liquid interface, resulting in lower γ values (Yano, 2012). Similar results were also obtained for solutions of whey protein concentrate (WPC; γ = 43.9–48.1 mN/m) (Fig. 1).

The γ of AL solutions (FG:AL ratio = 0:100) was measured over a range from 56.6 to 68.4 mN/m, depending on the pH (Fig. 1). These values were higher than those of FG solutions, but lower than those of buffer solutions (about 73 mN/m), indicating that AL had a low level of surface activity, although the activity level was much lower than that of FG. The low surface activity of AL is likely due to contamination with small amounts of surface-active proteins (Dickinson, 2003). The protein content of AL used in the present study was determined to be 3.4%, as mentioned in Section 2.1. Values of γ showed a decreasing trend with decreasing pH; as the pH decreased, mannuronic and guluronic acid residues within AL molecules (pKa = 3.38 and 3.65, respectively) became more protonated (Haug, 1961). Protonation could reduce the negative charge of AL chains, weakening intermolecular electrostatic repulsion at the surface that would result in better surface adsorption of AL molecules and thus reduce γ (Yang, Chen, & Fang, 2009).

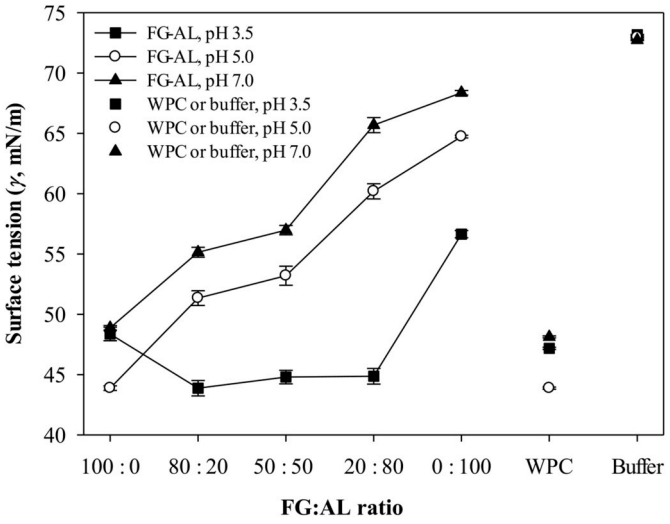

Fig. 1. Surface tension (γ) of aqueous FG − AL mixtures prepared at different values of FG:AL ratio (100:0, 80:20, 50:50, 20:80, and 0:100) and pH (3.5, 5.0, and 7.0) at an air-liquid interface of 25 °C. Values of γ for WPC and buffer solutions prepared at different pH values were also determined.

The γ of FG − AL mixtures was measured over a range from 43.9 to 65.7 mN/m and was influenced by both pH and the FG:AL ratio (Fig. 1). At a fixed value of FG:AL ratio, values of γ were significantly reduced with decreasing pH. At pH 7.0 (which is above the isoelectric point of FG), both biopolymers were negatively charged and thus repulsed each other, which could reduce the biopolymer surface coverage. However, at pH 3.5 (which is below the isoelectric point of FG), the two biopolymers were oppositely charged and were thus electrostatically attracted (Razzak et al., 2016). Charge neutralisation by electrostatic attractions between the biopolymers could increase biopolymer surface adsorption, which would reduce γ. At a fixed pH, values of γ became significantly greater (less surface active) as the AL fraction increased from 20% (FG:AL ratio = 80:20) to 80% (FG:AL ratio = 20:80) at both pH 5.0 and 7.0, but showed only a marginal increase at pH 3.5. When the pH was either 5.0 or 7.0, increasing the fraction of AL (a polyanion with poor surface activity) increased the electrostatic repulsion between negatively charged biopolymers, reducing the biopolymer surface coverage and increasing γ values. When the pH was 3.5, however, AL molecules electrostatically interacted with net-positively charged FG molecules; this charge compensation could reduce intermolecular electrostatic repulsion and cause dense packing of biopolymers at the surface, resulting in low γ values (43.9–44.9 mN/m), which were even lower than those for FG solutions (FG:AL ratio = 100:0) and WPC solutions under corresponding conditions. This effect was regardless of FG:AL ratios between 80:20 and 20:80. Previously, Miquelim et al. (2010) reported that γ values of egg albumin solution (at pH 3) were significantly reduced upon the addition of κ-carrageenan (egg albumin:κ-carrageenan = 91:9 (w/w); 1.65% total biopolymer concentration), likely due to the formation of complex coacervates (i.e. liquids-state electrostatic complexes) between the two biopolymers.

3.1.2. Electrophoretic mobility

The electrophoretic mobility (μ; the drift speed of a particle per applied electric field) of FG solutions (FG:AL ratio = 100:0) was measured to be 1.0 mm cm/V s at pH 3.5, where FG molecules were net-positively charged, and −1.1 mm cm/V s at pH 7.0, where FG molecules were net-negatively charged (Fig. 2). Values were close to zero at pH 5.0, which is near the isoelectric point of FG (5.4 at 25 °C) (Razzak et al., 2016). This supports the argument made in Section 3.1.1 that lower γ values for FG solutions at pH 5.0 compared to pH 3.5 or 7.0 (Fig. 1) can be attributed to weaker intermolecular electrostatic repulsion at pH 5.0, which leads to higher packing of FG molecules at the surface. Similar trends were observed for the μ values of WPC solutions (μ = −1.4 − 0.76 mm cm/V s) (Fig. 2).

The μ of AL solutions (FG:AL ratio = 0:100) was measured in a more negative range, from −3.8 to −6.2 mm cm/V, depending on the pH (Fig. 2). Values of μ became closer to zero as the pH decreased due to the increased protonation of mannuronic and guluronic acid residues within AL molecules (pKa = 3.38 and 3.65, respectively) (Haug, 1961). This supports the argument made in Section 3.1.1 that lower γ values at lower pH (Fig. 1) are due to decreased electrostatic repulsion between more protonated AL molecules at the surface.

The μ of FG − AL mixtures was measured in the range between values measured for FG and AL solutions (Fig. 2). As the pH was lowered from 7.0 to 3.5 at a given FG:AL ratio, the μ moved closer to zero due to higher charge neutralisation by increased electrostatic attractions between oppositely charged FG and AL molecules. Therefore, decreasing

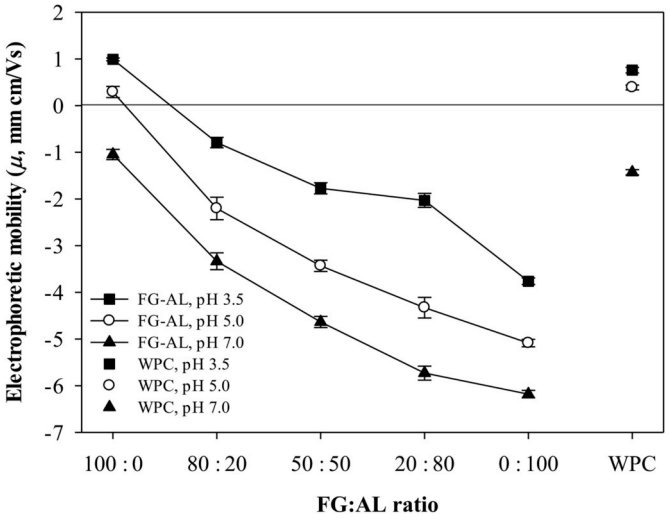

Fig. 2. Electrophoretic mobility (μ) of biopolymers and their complexes in aqueous FG − AL mixtures prepared at different values of FG:AL ratio (100:0, 80:20, 50:50, 20:80, and 0:100) and pH (3.5, 5.0, and 7.0) at 25 °C. Values of μ for WPC solutions prepared at different pH values were also determined.

the pH could reduce intermolecular electrostatic repulsion, resulting in better biopolymer surface coverage, which in turn results in a reduction of γ (i.e. more surface active), as discussed in Section 3.1.1. As the fraction of AL was raised from 20% (FG:AL ratio = 80:20) to 80% (FG:AL ratio = 20:80) at a given pH, the μ became more negative. This was due to an increase in the number of negatively charged AL molecules, which caused an increase in intermolecular electrostatic repulsion and thus an increase in γ (i.e. less surface active), as discussed in Section 3.1.1.

3.1.3. Apparent viscosity

The apparent viscosity (η) of FG solutions (FG:AL ratio = 100:0) was measured at low levels (1.08–1.15 cP) that were slightly higher than the viscosity of pure water (0.89 cP at 25 °C), and not significantly influenced by pH (Fig. 3). This is likely because FG molecules had a spherical aggregate structure rather than a fibril structure (Yang & Wang, 2009). Similar results were obtained for WPC solutions (η = 1.28–1.38 cP) (Fig. 3).

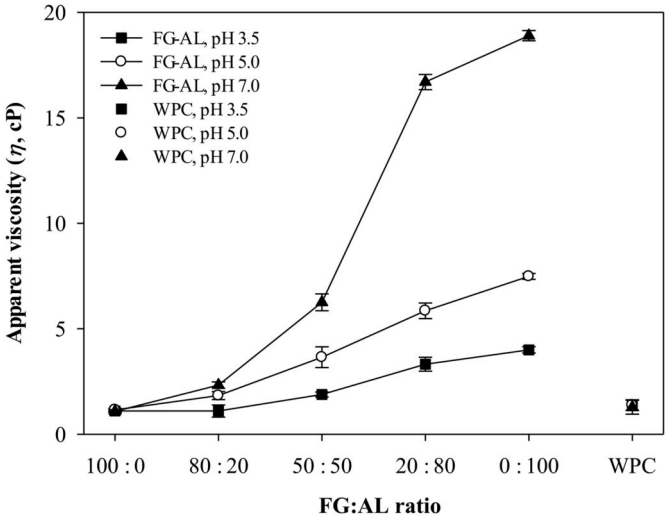

Fig. 3. Apparent viscosity (η) of aqueous FG − AL mixtures prepared at different values of FG:AL ratio (100:0, 80:20, 50:50, 20:80, and 0:100) and pH (3.5, 5.0, and 7.0) at 25 °C and a shear rate of 79.2 s^{-1}. Values of η for WPC solutions prepared at different pH values were also determined.

The η of AL solutions (FG:AL ratio = 0:100) was measured over a range of 4.00–18.90 cP, which was much higher than of FG solutions (Fig. 3). Values of η significantly increased with pH; as the pH increased, AL molecules became more negatively charged (due to the deprotonation of carboxylic residues) and thus more repulsive to each other. This could cause AL chains to further extend, leading to increased chain rigidity and water binding ability, and thus an increase in η (Draget, Moe, Skjåk-Bræk, & Smidsrød, 2006).

The η of FG − AL mixtures was measured over a range between the values of FG and AL solutions (Fig. 3). At a fixed FG:AL ratio, smaller values of η were obtained at a lower pH. As the pH decreased, FG and AL molecules were more strongly electrostatically attracted to each other and thus formed more charge-neutralised FG − AL complexes. This caused less stretching of the biopolymer chains and less intermolecular electrostatic repulsion, leading to a reduction in η. At a fixed pH, values of η increased as the fraction of AL shifted from 20% (FG:AL ratio = 80:20) to 80% (FG:AL ratio = 20:80); this was due to an increase in the number of negatively charged AL molecules, which boosted the extension and water affinity of the biopolymer chains, as discussed above (Draget et al., 2006). Generally, smaller value of η corresponded to greater surface activity (lower γ) and higher charge neutralisation (μ closer to zero) because it was related to the formation of FG − AL complexes by electrostatic charge compensation.

3.1.4. Particle size

The polydispersity index (PDI) of FG and AL solutions ranged between 0.2 and 0.4 and between 0.7 and 1.0, respectively (Table 1). This indicates moderate size distributions of FG solutions and rather broad size distributions of AL solutions according to the criteria by Bhattacharjee (2016). The PDI of FG − AL mixtures was 0.8 at pH 7.0 but decreased to 0.2–0.6 as the pH decreased to 5.0 and 3.5 (Table 1), implying that the size distribution became more narrow with the pH decrease probably due to the formation of soluble and insoluble electrostatic FG − AL complexes (Razzak et al., 2016). The solutions of FG, AL, and WPC showed one major peak in intensity-weighted size distribution regardless of pH, while FG − AL mixtures showed one major

Table 1
Polydispersity index (PDI) and percentage of each peak in intensity-weighted size distribution of biopolymers and their complexes in aqueous FG − AL mixtures prepared at different values of FG:AL ratio (100:0, 80:20, 50:50, 20:80, and 0:100) and pH (3.5, 5.0, and 7.0) at 25 °C. Values of WPC solutions prepared at different pH values were also determined.

Samples		PDI	Peak 1 (%)	Peak 2 (%)	Peak 3 (%)
pH	FG:AL ratio				
3.5	100:0	0.4 ± 0.1 ^{cde}	96 ± 6 ^{ab}	4 ± 6 ^{cd}	–
	80:20	0.6 ± 0.2 ^{abcd}	100 ± 0 ^{a}	–	–
	50:50	0.6 ± 0.0 ^{abcd}	100 ± 0 ^{a}	–	–
	20:80	0.6 ± 0.0 ^{bcde}	100 ± 0 ^{a}	–	–
	0:100	0.9 ± 0.2 ^{ab}	93 ± 2 ^{ab}	7 ± 2 ^{cd}	–
	WPC	0.8 ± 0.0 ^{abc}	100 ± 0 ^{a}	–	–
5.0	100:0	0.4 ± 0.0 ^{de}	91 ± 1 ^{ab}	6 ± 1 ^{cd}	3 ± 0 ^{a}
	80:20	0.2 ± 0.1 ^{de}	77 ± 5 ^{c}	23 ± 5 ^{b}	–
	50:50	0.3 ± 0.0 ^{de}	58 ± 1 ^{d}	40 ± 4 ^{a}	2 ± 0 ^{a}
	20:80	0.4 ± 0.1 ^{cde}	54 ± 3 ^{d}	46 ± 3 ^{a}	–
	0:100	0.7 ± 0.1 ^{abc}	88 ± 3 ^{abc}	12 ± 3 ^{bc}	–
	WPC	0.3 ± 0.0 ^{de}	100 ± 0 ^{a}	–	–
7.0	100:0	0.2 ± 0.1 ^{de}	85 ± 9 ^{bc}	12 ± 3 ^{cd}	3 ± 0 ^{a}
	80:20	0.8 ± 0.2 ^{ab}	89 ± 3 ^{abc}	11 ± 2 ^{cd}	–
	50:50	0.8 ± 0.1 ^{abc}	87 ± 3 ^{bc}	11 ± 6 ^{cd}	2 ± 0 ^{a}
	20:80	0.8 ± 0.1 ^{ab}	100 ± 0 ^{a}	–	–
	0:100	1.0 ± 0.0 ^{a}	100 ± 0 ^{a}	–	–
	WPC	0.3 ± 0.0 ^{de}	95 ± 0 ^{ab}	5 ± 0 ^{d}	–

Significant letters in the same column were obtained from ANOVA and Turkey's HSD at $p < 0.05$.

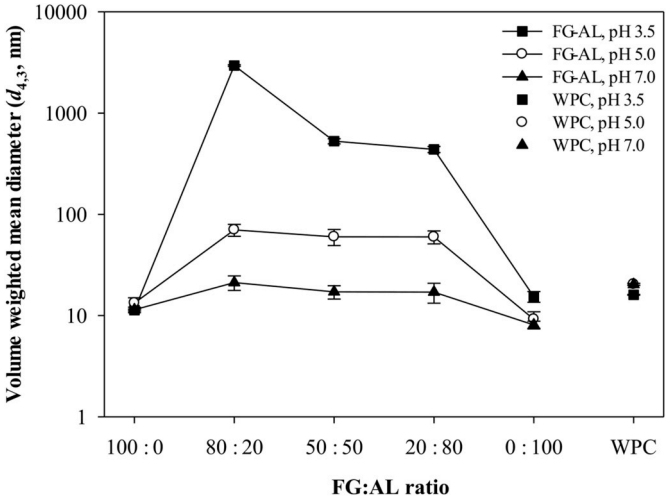

Fig. 4. Volume weighted mean diameters ($d_{4,3}$) of biopolymers and their complexes in aqueous FG – AL mixtures prepared at different values of FG:AL ratio (100:0, 80:20, 50:50, 20:80, and 0:100) and pH (3.5, 5.0, and 7.0). Values of $d_{4,3}$ for WPC solutions prepared at different pH values were also determined.

peak at pH 3.5 and 7.0 but two major peaks at pH 5.0 (Table 1). At pH 7.0, most biopolymer molecules in FG – AL mixtures are expected to exist in free soluble forms, showing one type of size category. At pH 5.0, FG – AL mixtures could contain not only individual biopolymers in free soluble forms but also soluble FG – AL complexes formed by weak electrostatic attractions (Razzak et al., 2016), which could generate two types of size category. At pH 3.5, FG and AL molecules existed mostly as insoluble complexes formed by strong electrostatic attractions (Razzak et al., 2016) and showed one type of size category.

The volume-weighted mean diameters ($d_{4,3}$) of FG molecules (FG:AL ratio = 100:0) was measured to be 11–13 nm; slightly larger values ($d_{4,3}$ = 16–20 nm) were measured for WPC (Fig. 4). No significant pH effect was observed relative to protein size.

The $d_{4,3}$ of AL molecules (FG:AL ratio = 0:100) was measured to be 8–9 nm at pH 5.0 and 7.0. This value was a little smaller than the values of FG (11–13 nm) and WPC (20 nm) in spite of the larger molar mass of AL (233.4 kDa) compared to those of FG (56 kDa) and WPC (20.9 kDa). This is probably because the hydrodynamic diameter (d_H) used for the determination $d_{4,3}$ is influenced not only by molar mass but also by many other factors, including interactions between biopolymers, molecular mobility, temperature, and medium properties such as pH, ionic strength, and viscosity. The $d_{4,3}$ of AL increased to 15 nm when the pH was reduced to 3.5. Razzak et al. (2016) also showed that the $d_{4,3}$ of AL increased from 6 to 10 nm as the pH was reduced from 5.5 to 3.8 at an AL concentration of 0.05% (and at 25 °C). As the pH decreased, AL molecules became more protonated, which in turn weakened intermolecular electrostatic repulsion and increased the relative strength of intermolecular hydrogen bonding, causing AL molecules to aggregate (Razzak et al., 2016).

The $d_{4,3}$ of FG – AL mixtures was measured over a wide range between 17 and 2900 nm (Fig. 4). Values of $d_{4,3}$ were larger than those for pure FG or AL molecules, and were strongly influenced by pH; very large values obtained at pH 3.5 (440 – 2900 nm) were primarily due to the formation of insoluble FG – AL electrostatic complexes, while size increases at pH 5.0 (60–70 nm) and 7.0 (17–21 nm) were caused by the formation of soluble FG – AL complexes (Razzak et al., 2016). The effect of the FG:AL ratio was not as strong as that of pH. Nevertheless, the largest size was measured when FG:AL ratio = 80:20 at all pH values. Roughly, larger size was related to stronger surface activity (lower γ), as seen by comparing Figs. 1 and 4.

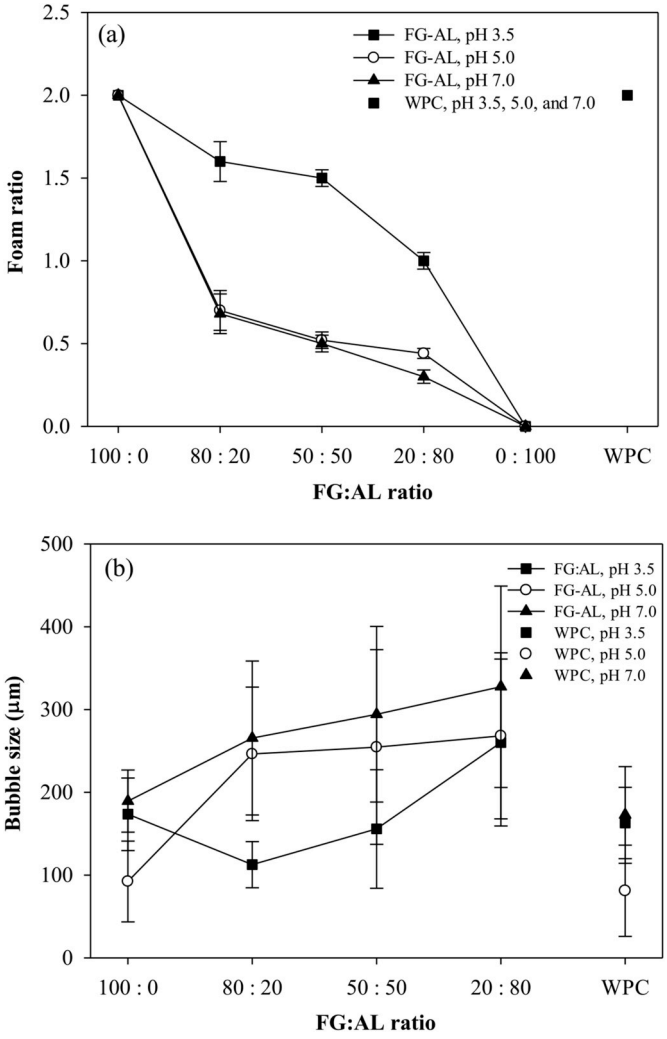

Fig. 5. (a) Foam ratio (FR) and (b) bubble size of freshly made FG – AL foams prepared at different values of FG:AL ratio (100:0, 80:20, 50:50, 20:80, and 0:100) and pH (3.5, 5.0, and 7.0) at 25 °C. Values for WPC foams prepared at different pH values were also determined.

3.2. Properties of foams

3.2.1. Foaming ability

For freshly made foams, those prepared with only FG (FG:AL ratio = 100:0) or WPC showed the highest FR values, and no foam was created by AL solution (FG:AL ratio = 0:100) (Fig. 5a). The high FR of FG solution decreased significantly when 20% of the FG was replaced with AL; it further decreased when the fraction of AL was increased (for all pH levels tested). This indicates that replacing FG with AL had an adverse impact on foam formation. Bubble size ranged from 92 to 189 μm for FG foams, and from 81 to 173 μm for WPC foams (Fig. 5b). Bubble size increased when FG was replaced with AL (except when foams were prepared at pH 3.5), and increased with AL fraction across all pH levels tested. That AL had an adverse impact on foaming ability may be attributed to its poor surface activity and highly viscous nature; the air-liquid surface tension (γ) increased as FG was replaced with AL (Fig. 1), and the apparent bulk viscosity (η) also increased with AL fraction (Fig. 3), which could cause the incorporation of air during foam formation to be more difficult (Makri & Doxastakis, 2007).

FG – AL foams prepared at pH 3.5 showed much higher FR values and smaller bubble sizes (especially when FG:AL ratio = 80:20 and 50:50), and thus higher foaming ability than foams prepared at pH 5.0 or 7.0 (Fig. 5). Additionally, when prepared at pH 3.5, FG – AL foams

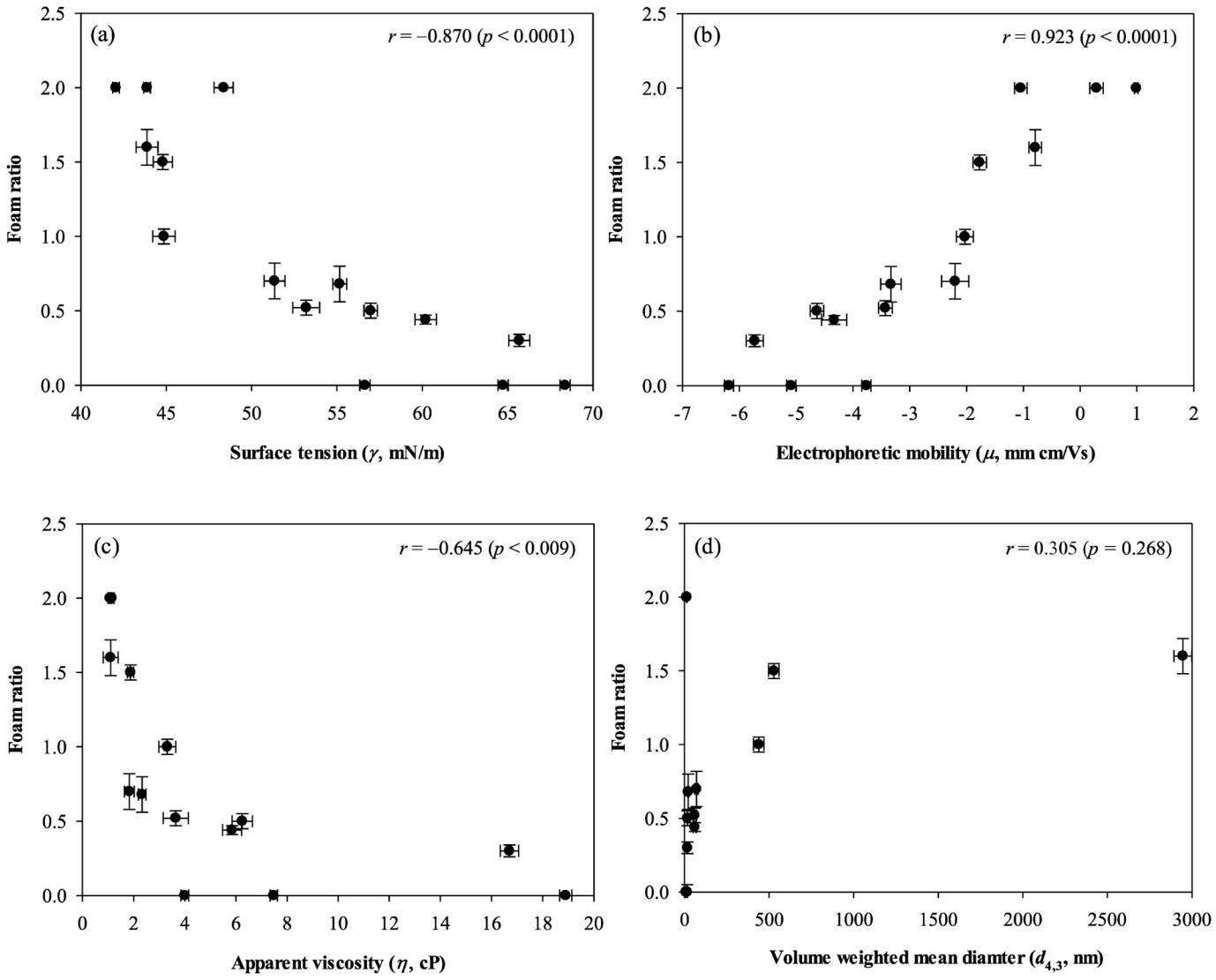

Fig. 6. Correlation between the foam ratio (FR) and four FG − AL mixture properties, including (a) surface tension (γ), (b) electrophoretic mobility (μ), (c) apparent viscosity (η), and (d) volume weighted mean diameter ($d_{4,3}$). Data were obtained from aqueous FG − AL mixtures prepared at different values of FG:AL ratio (100:0, 80:20, 50:50, 20:80, and 0:100) and pH (3.5, 5.0, and 7.0), all at 25 °C.

prepared with 20% AL had a significantly smaller bubble size than foams prepared only with FG (Fig. 5b). As the pH was lowered to 3.5 (below the isoelectric point of FG), electrostatic attractions between FG and AL molecules (and the resulting charge neutralisation) became stronger, thus promoting surface adsorption and network formation of the biopolymers, leading to a reduction in γ (see Section 3.1.1), as well as a reduction in η (see Section 3.1.3) that allowed a relatively large amount of air to enter the system during foam formation.

We closely examined correlations between the FR and four properties of biopolymer mixtures and solutions, including air-liquid surface tension (γ), electrophoretic mobility (μ), apparent viscosity (η), and particle size ($d_{4,3}$). Fig. 6a shows that the form ratio was significantly negatively correlated with γ ($r = -0.870$ at $p < 0.0001$); thus, foaming ability became stronger as the γ decreased. Fig. 6b shows that the FR increased linearly as the μ approached zero from negative values ($r = 0.923$ at $p < 0.0001$), showing that foaming ability was strongly related to charge neutralisation. Fig. 6c shows that there was a negative association between FR and η; i.e. foaming ability became stronger as the η decreased. The linearity of the relationship between FR and η was not high ($r = -0.645$ at $p < 0.009$), but became much higher when two data points obtained in the high viscosity region (> 16 cP) were not considered. No close correlation was found between FR and $d_{4,3}$ ($r = 0.305$ at $p = 0.268$) (Fig. 6d).

3.2.2. Foam stability

Foam stability was evaluated by measuring the FR as a function of storage time at 25 °C (Fig. 7). Foams prepared only with FG (FG:AL ratio = 100:0) or WPC showed the highest FR immediately after preparation (0 h, as described in Section 3.2.1), but quickly disappeared after 6 h at all pH levels tested. Foams prepared with FG − AL mixtures had lower FR values than freshly prepared FG or WPC foams (0 h, as discussed in Section 3.2.1), but showed much higher FR values during storage. This indicates that replacing a portion of FG with AL greatly enhanced long term foam stability while also suppressing the initial foam formation. This is likely because replacing FG with AL (a large, highly hygroscopic polysaccharide) increased not only bulk η, but also the ability to hold water molecules to form heavier and stiffer foams; it also enabled the formation of FG − AL network in the lamella and plateau border regions of the foams, which prevented the coalescence of air bubbles and reduced liquid drainage (Makri & Doxastakis, 2007; Miquelim et al., 2010; Żmudziński et al., 2014). FG − AL foams prepared at pH 3.5 (Fig. 7a) showed much higher stability; foams prepared

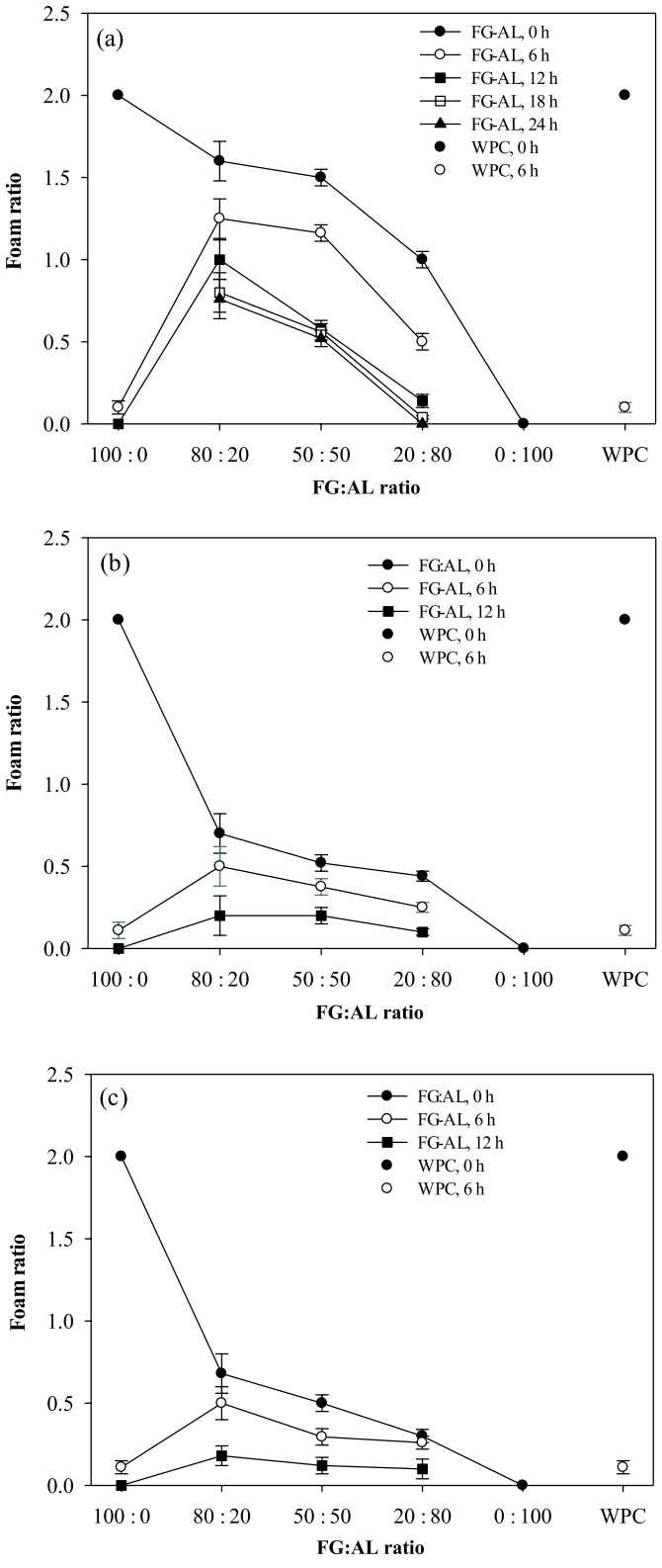

Fig. 7. Changes in foam ratio (FR) during storage at 25 °C for FG − AL foams prepared at (a) pH 3.5, (b) pH 5.0, and (c) pH 7.0 over different FG:AL ratios (100:0, 80:20, 50:50, 20:80, and 0:100). Changes for WPC foams prepared at different pH values were also determined.

at pH 3.5 (especially when FG:AL ratio = 80:20 or 50:50) showed high FR values even after 24 h, while foams prepared at pH 5.0 and 7.0 (Fig. 7b and c, respectively) disappeared quickly (and where no foam was observed after 18 h, regardless of the FG:AL ratio). There are likely reasons for this pH effect. Firstly, the γ decreased when the pH decreased to 3.5 due to enhanced biopolymer surface adsorption and network formation. This resulted from stronger FG − AL electrostatic attractions, which in turn increased charge neutralisation (see Section 3.1.1.). Secondly, bubble coalescence and liquid drainage were more inhibited with decreasing pH due to the formation of denser biopolymer network in the lamellar and plateau border foam regions.

4. Conclusions

This study demonstrated that replacing a portion of FG with AL suppressed the formation of foams but greatly enhanced their stability. This was due to interactions between FG and AL molecules, which strongly depended on the pH and FG:AL ratio. Replacing FG with AL (which is a highly viscous anionic polysaccharide with poor surface activity) suppressed foaming ability; firstly by increasing air-liquid surface tension, and secondly by increasing bulk viscosity, which could impede the incorporation of air during foam formation. Introducing hygroscopic, high molar mass AL molecules enhanced foam stability; firstly by forming heavier and stiffer foams via increased water holding ability, and secondly by forming FG − AL network in the lamella and plateau border regions of the foams, which could prevent the coalescence of air bubbles and reduce the liquid drainage. When foams were prepared at pH 3.5 (below the isoelectric point of FG), AL showed the least suppressing effect on foaming ability by reducing surface tension and incorporating a relatively large amount of air to the system, while it exhibited the largest enhancement effect on foam stability by reducing surface tension and forming a denser FG − AL network in the lamellar and plateau border regions of the foams. The results showed that the performance of FG-based food foams can be greatly improved by the addition of AL, especially under acidic conditions, due to electrostatic attractions between FG and AL molecules. In addition, this study provides fundamental insight into the relationship between protein-polysaccharide interactions and foam characteristics.

Acknowledgments

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2015R1D1A1A01059269).

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