Foaming properties and air–water interfacial behavior of corn protein hydrolyzate–tannic acid complexes

Yong-Hui Wang¹ · Yuan Lin² · Xiao-Quan Yang²

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Abstract The complexation of corn protein hydrolyzate (CPH) with tannic acid (TA) was utilized to improve the foaming properties of CPH itself, and the air–water interfacial behavior of CPH–TA complex was also investigated. The results showed that the surface hydrophobicity of pure CPH was significantly decreased in bulk solution after the complexation with TA. Compared with pure CPH, the foams stabilized by CPH–TA complex showed higher interfacial thickness between the bubbles, which well explained the better long term stability of the corresponding foams. Therefore, the complexation maintained the good foaming capacity of CPH itself, but considerably increased its foam stability. Moreover, the air–water interfacial behavior study demonstrated that the complexation slightly decreased the interfacial activity of CPH itself, but considerably increased its interfacial viscoelasticity, suggesting more stable of the air–water interface stabilized by CPH–TA complex compared with that stabilized by CPH alone. These findings indicated that foaming properties of the surface active components were closely related with its air–water interfacial behavior. The

study suggested that CPH–TA complex could be used as a stabilizer in constructing the peptides-based foams.

Keywords Corn protein hydrolyzate · Tannic acid · Complexation · Air–water interface · Foaming properties

Introduction

Foams are colloidal systems in which tiny air bubbles are dispersed in an aqueous continuous phase (Walsh et al. 2008). Many processed food in daily contains a large number of foams, such as ice cream, whipped topping, beers, champagne, breads, and cakes et al. The foaming properties (e.g. foaming capacity and physical stability) are mainly rely on the properties of surface active components in foaming agents (Wan et al. 2014a). Compared with the chemical foaming agents, the food-grade one is more popular with consumers. Protein is the major type of food-grade surface-active substances in food processing due to the amphiphilic properties, as well as the nutrition and safety characteristics. It has been reported that during the foam formation protein could quickly adsorb at the air–water interface, decreasing surface tension, increasing the visco-elasticity property of the liquid phase, finally forming a stabilized air–water interfacial film (Zayas 1997). Therefore, protein could stabilize foams with longer time scales due to the formation of highly elastic networks on the bubble surface compared with the low-molecular weight surfactants (Wan et al. 2014a).

However, it should be noticed that not all the proteins possess the good functional properties (e.g. foaming and emulsification) due to their solubility problem. This especially true for some cereal proteins, e.g. corn protein and wheat gluten. The protein hydrolyzate deriving from the

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✉ Xiao-Quan Yang
fexqyang@scut.edu.cn

¹ Key Laboratory of Biomarker Based Rapid-Detection Technology for Food Safety of Henan Province, Food and Bio-Engineering College, Xuchang University, Xuchang 461000, People's Republic of China

² Department of Food Science and Technology, South China University of Technology, Guangzhou 510640, People's Republic of China

enzymolysis of native food proteins (e.g. soy protein, sunflower protein, wheat gluten) were utilized to improve the foaming properties of the native proteins (Agyare et al. 2009; Manoi and Rizvi 2009; Rodriguez Patino et al. 2007). Generally, mild enzymatic hydrolysis could obviously increase the foaming capacity of the plant proteins. However, the foam stability for protein hydrolyzate in long term is still poor. Hence, it is impossible for protein hydrolyzate, especially for that with high hydrolysis degree, as a role of foaming agents in food industry.

Tannic acid (TA) is a specific type of plant polyphenol, and it is widely found in gallnut plants, e.g. *Rhus semialata* or *Sicilian Sumac* leaves. Numerous of phenol groups in the structure result the high macular weight of TA itself. TA is generally recognized as safe by the Food and Drug Administration, and it could be used as a direct additive in food products. In fact, the application of TA in food industry is far more widespread and significant amounts of TA are used as the process aids in beer clarification, aroma compound in soft drinks and juices. TA contains sufficient hydroxyls and carbonyls, it could be easily form stable complex with other food macromolecules, e.g. proteins and polysaccharides (Balange and Benjakul 2009; Patel et al. 2013). It has been pointed out the protein–phenolic complex could rapidly concentrate at the O/W interface during emulsification, which not only maintained the surface-active nature of the protein, but kept the antioxidative activity of the phenolic compounds (Almajano and Gordon 2004). Therefore, appropriate non-covalent modification with TA is an efficient approach to obtain the modified protein with the higher interfacial activity. In previous study, we found that the complexation of zein hydrolyzate with TA could significantly improve the stability of the alga oil nano-emulsions (Wang et al. 2016a). However, little information on the air–water interfacial activity and foaming properties of their complex has been reported.

As a major by-product of corn wet milling, corn gluten meal (CGM) contains 50–60% (w/w) protein. It has been reported that CGM contains plenty of zein, it has the similar amino acid constitutions with zein (Lin et al. 2011). Despite the high protein content of CGM, poor water solubility makes the inferior function properties of itself. Hence, it is difficult to be used as a food ingredient for CGM in present. There is always a hot spot in food science field to explore the efficient approach to utilized CGM in food processing. It is well known that the solubility of corn protein could be hugely increased by enzymatic modification, but the obtained corn protein hydrolyzate (CPH) still have poor function properties due to the low molecular weight of the peptides in CPH. However, the complexation of CPH with TA may be a feasible approach to improve the function properties of CPH itself. Therefore, CPH was

selected as a model to investigate the impact of the complexation on the foaming properties of protein hydrolyzate, and the air–water interfacial behavior of the complex was also investigated to explore the relationships between foaming properties and air–water interfacial behavior.

Materials and methods

Materials

CGM was kindly granted by corn developing Co., Ltd. (Zhucheng, China). The protein content of CGM was 52.9 ± 1.1 g/100 g, determined by Kjeldahl method ($N \times 5.71$, wet basis). α -Amylase (3.5 AU/g) and Alcalase 2.4 L (endoproteinase from *Bacillus licheniformis*, 2.4 AU/g) were purchased from Novozymes biotechnology co., LTD. CPH was prepared from CGM according to our previous report (Wang et al. 2016b). The hydrolysis degree of CPH was 25%, and vast majority of the peptides were smaller than 1 kDa (supporting information). Tannic acid (> 99%), Nile Blue A (> 99%) were all obtained from Sigma-Aldrich (St. Louis, MO). All other chemicals used were of analytical grade.

Preparation of the CPH–TA complex

Stock solutions of CPH and TA (10 mg/mL) in PBs (pH 7.0, 10 mM) were prepared respectively under ambient condition, and then the solutions were mixed to obtain the mixture with the TA/CPH mass ratios of 0.1, 0.2, 0.3 and 0.5 respectively. As the control, pure CPH solution was also prepared. For all cases, the concentration of CPH was fixed in 5 mg/mL.

Zeta potential

Before the determination, the prepared CPH and CPH–TA solutions were diluted 5 times respectively using PBs (pH 7.0, 10 mM). Zeta potential of the diluted solutions was determined by a Zeta-sizer Nano (Malvern Instruments Ltd., Worcestershire, UK). Each value represents the average of at least three independent trials.

Fourier transform infrared (FTIR)

The freshly prepared CPH–TA solution (with the TA/CPH mass ratio of 0.3) was lyophilized (Dura-Dry MP freeze-dryer, FTS Systems, Inc., Ridge, NY) under light-resistant conditions by covering with foil paper before FTIR determination. As contrasts, the powdered samples of the pure CPH, TA and the CPH/TA physical mixture were also

prepared. FTIR spectra were recorded on a Nicolet Avatar 360 FTIR spectrometer.

Surface hydrophobicity determination

The surface hydrophobicity of pure CPH and CPH–TA complex in bulk solution were determined using a fluorescence probe, 1-anilinonaphthalene-8-sulfonic acid (ANS^-). The methodology was adapted according to Chen et al. (Chen et al. 2015). In brief, stock solutions of ANS^- (8×10^{-3} M) and CPH or the CPH–TA complex (1.5% (w/v)) was prepared in PBs (pH 7.0, 10 mM). The ANS^- solution (20 μL) was added to 4 mL of the buffer containing increasing amounts (0–50 μL) of 1.5% (w/v) sample solutions. The relative fluorescence intensity (FI) was measured at 25 °C, with the excitation and emission wavelengths of 390 and 470 nm respectively. For each sample, the initial slope of FI versus sample concentration plot was calculated by linear regression analysis and used as an index of surface hydrophobicity.

Foams fabrication and characterization

The foams from CPH and CPH–TA solutions were fabricated respectively using a handheld shearing machine (CR22G II, Hario Co. Ltd., Japan). The solutions (15 mL) were placed in the glass tubular containers (inside diameter 31 mm, height 98 mm). The stirring head was placed in the center of the container, and maintaining 10 mm below the air–water interface. The stirring process was maintained 1 min under ambient condition. The maximum foam volume was recorded immediately after the stirring, and the foam volume at varying time was also recorded to evaluate the stability of the foams. The foam volume was calculated through the whole volume minus the liquid volume. For each solution, the concentration of CPH was set at 5 mg/mL.

CLSM-imaging was used to observe the microstructure of the foams. Firstly, pure CPH solution and CPH–TA solution (with the TA/CPH mass ratio of 0.3) was labeled by Nile Blue A (1 mg/mL), and then the foams were fabricated according to the previous procedure. Before the observation, a small amount of the labeled foam was placed on the concavity slide, and the cover glass was put on the slide. CLSM-images of the foams were recorded with a Leica TCS SP5 Confocal Laser Scanning Microscope (Leica Microsystems Inc., Heidelberg, Germany), equipped with an inverted microscope (Model Leica DMI6000), and the excitation wavelength was set at 633 nm.

Characterization of the interfacial behavior

The dynamic interfacial behavior of pure CPH and the CPH–TA complex at the air–water interface were monitored by recording temporal evolution of surface pressure and surface dilatational parameters using an optical contact angle meter (OCA-20, DataPhysics Instruments GmbH, Germany) equipped with oscillating drop accessory (ODG-20). The details of this equipment was described by Tamm et al. (2012). All the experiments were carried out at 25 °C. For CPH, TA and CPH–TA solutions, the concentration of CPH was all set at 5 mg/mL.

Dynamic interfacial adsorption was determined by monitoring the evolution of surface tension. The methodology was adapted according to Wan et al. (2014a) with minor modification. A drop of the sample solution (15 μL) was allowed to stand for 60 min to monitor the adsorption process at the air–water interface. An image of the drop was continuously recorded by a CCD camera and digitalized. The surface tension (γ) was calculated through the shape analysis of a pendant drop. The surface pressure is $\pi = \gamma_0 - \gamma$, where γ_0 (72.5 ± 0.5 mN/m) is the surface tension of phosphate buffer (10 mM, pH 7.0) and γ is the time-dependent surface tension of the tested sample.

To obtain the parameters of surface dilatational rheology, sinusoidal interfacial compression and expansion were performed. Details of the methodology were given by Wang et al. (2012). The drop volume at 10% of deformation amplitude and 0.1 Hz of angular frequency were selected. The drop (10 μL) was subjected to repeated measurements of 5 sinusoidal oscillation cycles followed by a time corresponding to 30 cycles without any oscillation up to 50 min. The surface viscoelastic parameters, including surface dilatational modulus (E), surface elastic (E_d), and surface viscous (E_v), were derived from the change in γ . E is a complex quantity and composed of real and imaginary parts ($E = E_d + iE_v$). The real part of the dilatational modulus or storage component is the dilatational elasticity (E_d). The imaginary part of the dilatational modulus or loss component is the surface dilatational viscosity (E_v).

Air–water interface morphology observation

The Langmuir trough (KSV liquid–liquid trough, Tietäjäntie 2, FI-02130 Espoo, Finland) was used in this work to obtain the air–water interfacial films, and the details of this apparatus was reported by Zou et al. (2017). The Langmuir trough was equipped with an automatic dipper for Langmuir–Blodgett (LB) films deposition onto solid substrates. When the surface pressure reached 20 mN/m, the LB films constituted by pure CPH or CPH–TA complex (with the TA/CPH mass ratio of 0.3) was transferred on a mica plate

of dimensions 20 mm × 30 mm at a constant velocity of 2 mm/min by a vertical pulling. The morphology of the LB films was observed by using Field Emission Scanning Microscope (FE-SEM, Zeiss, Oberkochen, Germany). The mica plates deposited with the films were loosely glued onto a conductive adhesive mounted on a stainless steel stage, subsequently coated with a thin conductive gold and platinum layer using a sputter coater (Hummer XP, Anat-ech, Union City, CA, USA).

Statistical analysis

Unless otherwise specified, all measurements were carried out in triplicate, and an analysis of variance (ANOVA) of the data was performed using the SPSS 19.0 statistical analysis system. The Duncan Test was used for comparison of mean values among the three treatments using a level of significance of 5%.

Results and discussion

Complexation of CPH with TA and the surface hydrophobicity

The surface zeta potential of pure CPH and CPH–TA in bulk solution is shown in Fig. 1a. For pure CPH, the zeta potential was only −11.3 mV. For CPH–TA, the zeta potential was considerably increased, and the values were between −25 mV and −28 mV. However, there were no obvious correlations between the zeta potential and the concentration of TA. This result may be attributed to the sufficient carboxyls of TA, and the complexation process resulted in CPH embed in the core of the formed CPH–TA complex. As seen from the inset of Fig. 1a, at the constant CPH concentration (0.5 wt%), the turbidity was increased with the proportion increasing of TA. It has been reported that the increasing of turbidity can be considered as a complexation process (Patel et al. 2013). Hence, in this case the increase in turbidity should be attributed mostly to the micro-aggregation of the complexation between CPH and TA.

FTIR spectra of pure CPH, TA, physical mixture of CPH and TA, and CPH–TA complex are shown in Fig. 1b. It can be observed that the characteristic absorption peak (3486 cm^{-1}) of hydroxyl for pure TA was obviously shifted to 3407 cm^{-1} after the complexation with CPH. This may be resulted from the hydrogen bonding interaction between CPH and TA. This speculation can be well supported through the previous report, where Zein/TA complex colloidal particles were fabricated by their hydrogen bonding (Zou et al. 2015). For CPH–TA complex, the two peaks (2961 cm^{-1} and 2875 cm^{-1}),

indicating the C–H stretching vibration for pure CPH, were all disappeared or obviously shifted. As to our knowledge, C–H groups are generally located in the hydrophobic side of the peptides. Hence, the hydrophobic interaction between TA and CPH also take place in the complexation process.

The structure of the CPH–TA complex could also be reflected by the surface hydrophobicity determination (Fig. 1c). It can be observed that the addition of TA result the surface hydrophobicity of CPH significantly decreased ($p < 0.05$), and the influence was strengthened with the concentration increase of TA. This observation indicated the reduction of surface hydrophobic site for CPH after the complexation with TA. This result well confirmed the previous speculation that CPH was located in the hydrophobic core of the formed CPH–TA complex, and TA was distributed to the surface of the complex. Interestingly, this result was inconsistent with our previous report (Wang et al. 2016a), in which the complexation of zein hydrolyzate (ZH) and TA was confirmed by fluorescence titration and isothermal titration calorimetry, but the structure of the formed ZH–TA complex was supposed as ZH “wrapping around” TA, and TA was in the core of the ZH–TA complex. From our perspective, this contradiction may be caused by the differences of hydrolysis degree and concentration, but the specific mechanisms need to be further investigation.

Foaming properties

Appearances of the foams stabilized by pure CPH and CPH–TA complexes with varying TA/CPH mass ratios are shown in Fig. 2a. In all cases, good foaming capacity could be observed for the freshly prepared foams. However, it could be noticed that the addition of TA reduced the foaming capacity for CPH solution. Foams are thermodynamically unstable, and their relative stability is governed by factors such as liquid drainage, coarsening, and coalescence (Wan et al. 2014a). Therefore, it could be observed in long term, despite the best foaming capacity for pure CPH, its foam stability was considerably poor, showing serious instable phenomenon, including bubble drop-off rate, coarsening and coalescence. According to the previous report, similar behavior could be observed in the foams stabilized by many LMW surfactants (Ruízhenestrosa et al. 2008). Hence, pure CPH has the similar characteristics with the LMW surfactants in foams stabilization due to the low molecular weight (supporting information). However, for the foams stabilized by CPH–TA complex (except for the case with the TA/CPH mass ratio of 0.1) all showed good foam stability, and the foams could maintain a delicate appearance over 2 h under the ambient condition.

Fig. 1 **a** Zeta potential of CPH and CPH-TA complex. Inset: appearances of the corresponding solutions of CPH and CPH-TA complex; **b** FTIR spectra of CPH, TA, CPH/TA physical mixture, and

the CPH-TA complex; **c** The surface hydrophobicity of pure CPH and CPH-TA complex, the different letters above the bars mean significant differences between the group ($p < 0.05$)

To further evaluate the foam stability, the maximum foam volume and the foam volume versus time after foam generation were measured respectively (Fig. 2b, c). The maximum foam volume was decreased from 56 to 47 mL with the concentration increasing of TA, and it was very consistent with the appearances of the freshly prepared foams. From the curve of foam volume as a function of time, it can be seen the volume of pure CPH is rapidly decreased. However, for the foams stabilized by CPH-TA complex (with the TA/CPH mass ratio of 0.2, 0.3 and 0.5 respectively), despite the volumes were also decreased at the initial 10 h, but the volumes were quite stable in longer time scale. This result indicated that the foams stabilized by CPH-TA complex (especially for the case with the TA/CPH mass ratio of 0.3) showed high stability in long term storage, which may have good application prospect in the foam food processing (e.g. ice cream, whipping cream, et al.).

Moreover, the differences in micro-structure for the foams stabilized by pure CPH and CPH-TA complex could be well observed by the CLSM images (Fig. 3). For pure CPH stabilized foams, the adsorption layer around the bubbles was thin and the color was light, indicating only few CPH present at the air–water interface. However, the adsorption layer consisted by CPH-TA complex was quite thick and color was dark, which suggested that more CPH adsorbed to the air–water interface around the bubbles. Therefore, this result could further confirm the observation that CPH-TA complex have better foam stability compared with CPH alone.

Air–water interfacial behavior

The interfacial pressure and interfacial dilatational elasticity were investigated to investigate the relationship between air–water interfacial behavior and foaming properties. The time evolution of the interfacial pressure at the

Fig. 2 **a** Appearances of the foams as a function of time for the solutions of pure CPH and CPH–TA complex; **b** Maximum foam volume obtained from the solutions of CPH and CPH–TA complex,

the different letters above the bars mean significant differences between the group ($p < 0.05$); **c** Foam volume as a function of time for the corresponding solutions of CPH and CPH–TA complex

Fig. 3 CLSM images of the bubbles stabilized by CPH (**a**) and the CPH–TA complex (**b**)

air–water interface is presented in Fig. 4a. The interfacial pressure for pure CPH, TA and CPH–TA were all increased with adsorption time increasing, which can be associated with the surface-active substances adsorption at the air–water interface. This observation is very in line

with the previous report (Wan et al. 2014b). The π values for CPH was obvious higher than TA, which means CPH have better air–water interfacial activity compared with TA. Moreover, it can be noticed that pure CPH and CPH–TA have similar π – t curve, but the complexation was still obviously reduced the π values of CPH, and this tendency was more obvious with the concentration increase of TA. This observation indicated that the complexation reduced the transfer rate of CPH from the bulk to air–water interface, and it may be attributed to the increased size as well as the decreased surface hydrophobicity of the complex compared with pure CPH, which increased the sorption barrier. Furthermore, it could be noticed that this observation is very in line with the previous study that pure CPH have the best foaming capacity compared with the CPH–TA complexes (Fig. 2a, b).

The graph of dynamic dilatational elasticity (E_d) against adsorption time for pure CPH and CPH–TA complex are

Fig. 4 **a** Interfacial pressure (π) of the air–water interfacial adsorption layers formed from TA, CPH and CPH–TA complex; **b** Time-dependent air–water interfacial dilatational elasticity (E_d) of the

adsorbed layers formed from CPH and CPH–TA complex; **c** Surface dilatational modulus (E) as a function of interfacial pressure (π) for CPH and CPH–TA complex

shown in Fig. 4b. In all cases, the gradual increase in E_d with adsorption time should be associated with the adsorption of surface-active components at the interface. For pure CPH, the E_d (11 mN/m) was very low, and there was no obvious change in the whole adsorption time. However, for CPH–TA complex, except the case with the TA/CPH mass ratio of 0.1, the E_d were rapidly increased with adsorption time increasing, especially true for the complex with the TA/CPH mass ratio of 0.3. The result suggested that the interfacial films constituted by CPH–TA complex were the strong viscoelastic interfacial networks, which have a good potential to against the interfacial turbulence. Hence, this observation well confirmed that the complexation with TA has positive effect on improving the interfacial stability of CPH, and the result is very accordance with the previous foam stability study (Fig. 2c). Moreover, it can be noticed that when the TA/CPH mass ratio was more than 0.3, E_d was decreased due to the

decrease of surface hydrophobicity of the CPH–TA complex.

Figure 4c presents the evolution of surface dilatational modulus (E) as a function of interfacial pressure (π) for CPH and CPH–TA complex. In all cases, E values were increased almost immediately with π increasing, revealing the increasing of the interactions between the adsorbed materials at the air–water interface, in agreement with the theory of Lucassen-Reynders (1974). The higher E and π could be attributed to more CPH–TA complex adsorbed at the interface, and the interface stabilized by the complex (especially for the case with high TA/CPH mass ratio) could have higher thickness compared with that stabilized by pure CPH. This result could well support the previous observation that the foams stabilized by CPH–TA complex have thicker adsorption layer compared with that stabilized by pure CPH (Fig. 3). Furthermore, the slope (2.3–9.5) of E – π curve all large than 1.0 indicated the adsorption process was non-ideal adsorption and the interactions was

strong for the adsorbed materials at the interface (Lucassen-Reynders 1974).

Observation of the adsorption layer

To visually observe the air–water interfacial topography, the air–water interfacial film was fabricated by Langmuir–Blodgett using the Langmuir trough, and the topography of the interfacial film was observed by FE-SEM (Fig. 5). It can be seen obviously that the aggregates of CPH formed at the interfacial film were considerably sparse and un-uniform. This observation means the adsorption layer constituted by CPH aggregates was very thin and unstable, and it was mainly due to the weak interactions between the peptides in CPH at the air–water interface. However, for the CPH–TA complex, the topography was completely

different. The air–water interfacial film constituted by the aggregates of CPH–TA complex exhibited an orderly and compact structure, indicating the strong interactions between the complexes at the interface. This result suggested that the interface stabilized by CPH–TA complex could possess strong stability, and it is very in line with the air–water interfacial behavior study.

Conclusion

In this study, the complexation of CPH and TA was successfully utilized to improve the foaming properties of CPH itself. The complexation process was mainly driven by hydrophobicity interaction and hydrogen bond binding. The surface hydrophobicity of CPH significantly decreased

Fig. 5 FE-SEM images of the air–water interfacial films stabilized by pure CPH (a) and CPH–TA complex (b)

after the complexation with TA. The results shown the complexation slightly decreased the interfacial activity of CPH itself, but considerably increased its interfacial viscoelasticity. Moreover, the study also indicated that the foaming properties were very accordance with the interfacial behavior investigation. Compared with CPH alone, the foams stabilized by the CPH–TA complex showed higher interfacial thickness between the bubbles, and they exhibited better long term stability. The complexation fully maintained the good foaming capacity of CPH itself, but considerably increased its foam stability. These findings suggest that CPH–TA complex could be utilized as a stabilizer in constructing the hydrolyzate-based foams and opens up the possibility of utilizing peptide-polyphenol complex as an efficient food-grade foaming agent in food industry.

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