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Interaction Between Iso-α-Acid Extracted from Hops and Protein Z Improves Beer Foam Quality and Stability

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Abstract: Foam quality is an important index for judging the quality of beer. In this experiment, the interaction between PZ and iso-α-acid extracted from hops and its effect on beer foam production were investigated. According to the results of fluorescence titration experiments, the stoichiometric number ratio of PZ interacting with iso-α-acid was 3.91 ± 0.39, and the binding constant K was (2.16 ± 0.23) × 10^5 M^{-1}. According to the results of molecular dynamics simulations, the binding sites of iso-α-acid in PZ were Leu-396, Ser-292 and Lys-290. The secondary structure of PZ was altered by the addition of iso-α-acid, and the percentage of β-sheets increased from 21.75% to 29.74%. which increased the protein's flexibility, leading to enhanced foaming, stability, and texture of the foam.

Keywords: protein Z; iso-α-acid; interaction; foam; beer

1. Introduction

Beer, one of the most consumed alcoholic beverages globally, is celebrated for its complex flavor profile and distinctive foam characteristics. Foam not only beautifies the appearance of the beer, but also enhances the flavor and taste of the beer to a certain extent. The interaction of carbon dioxide gas in the foam with the alcohol and esters in the beer is one of the most important features for consumers and brewers to judge the quality of beer [1]. Rich, stable and long-lasting foam is one of the most important characteristics of good beer. Good beer foam stability directly influences consumer preference and visual impression, which in turn influences the buyer's decision [2]. The foam not only enhances the sensory experience but also plays a crucial role in the retention of volatile compounds, thereby influencing the overall aroma and flavor of the beer [3–5]. The stability and persistence of beer foam are determined by the intricate interplay of various components, with proteins and iso-α-acids being key players in this dynamic equilibrium.

Iso-α-acids, derived from the isomerization of hop-derived α-acids, are known for their bittering properties and are integral to the beer's flavor profile [6]. The iso-α-acid content varies in different types of beer and hops. In the study by Wang et al., the iso-α-acid content in dry hops and malt beer was measured by HPLC and UPLCMS. The content of iso-α-acid in dry hops was in the range of (27.74 ± 3.2 to 74.88 ± 3.2 mg L^{-1}), and the content of iso-α-acid in malt beers was in the range of (7.20 ± 0.5 to 36.26 ± 2.5 mg L^{-1}). Iso-α-acids were indisputably the most prevalent hop bitter acid in beers regardless of beer style [7]. Despite its potential to significantly affect foam characteristics, PZ, a major component of beer foam, is known for its surface-active properties, which are critical for foam formation and stability [8–10].

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Recent studies have suggested that the interaction between iso-α-acid and protein Z could modulate the foam's physical properties, such as surface tension and viscosity, thereby affecting its persistence and stability [11]. The binding of iso-α-acid to protein Z may alter the protein's conformation, potentially leading to changes in its surface properties and its ability to stabilize the foam [12]. This interaction could be influenced by various factors, including the molar ratio of iso-α-acid to protein Z, the presence of other beer components, and the environmental conditions under which the beer is consumed. There have been many similar references in previous studies to the existence of an interaction between protein Z and humulinone, which has an effect on the stability of beer foam [13,14]. Iso-α-acid is formed by isomerization of α-acid in hops during wort boiling, humulinone is an oxidation product of α-acid, usually formed during hop storage or during dry hopping of beer, and is structurally similar to iso-α-acid, but is more polar and more soluble. However, the specific binding effect between iso-α-acid and protein Z is currently unclear. Therefore, we have carried out the following series of research work.

In this study, we aim to delve deeper into the molecular interactions between iso-α-acid and protein Z, and how these interactions influence the foam's persistence and stability in beer. By employing a combination of biochemical assays, spectroscopic techniques, and computational modeling, we seek to elucidate the binding affinity, stoichiometry, and structural changes associated with the protein Z–iso-α-acid complex. Our findings could provide valuable insights into the role of iso-α-acid in beer foam dynamics and potentially guide the development of brewing strategies to optimize foam quality.

2. Materials and Methods

2.1. Chemicals and Materials

Barley was purchased from commercial Malteurop (Baoding) in China. HPLC grade methanol and phosphoric acid were purchased from Sigma-Aldrich Limited, St. Louis, MI, USA, and hops extract was purchased from BarthHaas (Beijing, China). All reagents used for gel electrophoresis, 8-anilino-1-naphthalenesulfonic acid (ANS) and potassium bromide were purchased from Solarbio (Beijing, China). All other reagents used in the experiments were of analytical grade and ultrapure water was used throughout.

2.2. Preparation of PZ and Iso-α-Acids

PZ was isolated and purified from malt referred to Jiang's method [15]. First, deionized water (1:5, m/v) was added to crushed malt, mixed well, stirred at room temperature for four hours, and then filtered. After that, 40–60% ammonium sulfate solution was added, heated at 90 °C for half an hour, and the product was centrifuged at 10,972× *g* for ten minutes to obtain the supernatant. Proteins obtained in this way were loaded onto an anion exchange column, HiPrep 16/10 DEAE FF on an anion exchange column HiPrep 16/10 DEAE FF with the AKTA avant protein purification system (GE Healthcare, Pittsburgh, PA, USA), and the obtained proteins were dialyzed into 50.0 mM citrate (pH = 4.4) buffer after identification by SDS-PAGE and native PAGE.

Iso-α-acid was prepared referring to previous methods [16]. A sample of 200 g of supercritical carbon dioxide hop extract (α-acids 55.6% (*w*/*w*) and β-acids 22.6% (*w*/*w*)) was dissolved in hexane (1.5 L), and partitioned with 0.24 M disodium carbonate (2 L). In this process, α-acids were selectively extracted into the aqueous solution. The aqueous solution was acidified with 6 N HCl, and free α-acids were extracted with hexane. After being washed with saturated NaCl, the hexane layer was dried over anhydrous sodium sulfate and concentrated to dryness to give the α-acid fraction (105 g). HPLC analysis confirmed that the purity of the α-acid (sum of iso-α-acid) in this fraction met the experimental requirements.

2.3. Fluorescence Titration

Fluorescence titration techniques have been widely used to study protein–small molecule interactions. For example, Dai et al. studied the interaction of lactoferrin and tannic acid [17], Lv et al. investigated the interaction of NADH with pea seed ferritin [18], and Jiang et al. investigated the binding of curcumin to barley protein Z. These studies provide important structural and kinetic information [15]. Fluorescence titration was conducted with minor adjustments to the technique previously reported. The titration process utilized a Cary Eclipse fluorescence spectrometer from Varian, USA. The procedure involved the sequential addition of 1 μL of a 4 mM iso-α-acid solution into 1mL of a 4 μM PZ solution. After thorough mixing for a duration of 5 min, the fluorescence was measured. The setup included an excitation wavelength set at 280 nm, with the detection wavelength sweep ranging between 290 to 500 nm. The excitation and emission slits were configured at widths of 5 nm and 10 nm, respectively.

2.4. Dynamic Surface Tension

With reference to Maeda K. et al. [19], the Wilhelmy approach was employed to determine the surface tension (γ) of the sample solution, utilizing a surface tensimeter model DCAT21 from Dataphysics in Filderstadt, Germany. The variation in γ was continuously tracked over a period of 3600 s.

2.5. Fourier-Transform Infrared Spectroscopy

Referring to the research line of Meng Y. et al. and Sui et al. [20,21]. The Fourier-transform infrared (FTIR) spectroscopy data were acquired using a Thermo Nicolet iS50 FTIR spectrometer, manufactured by Thermo Nicolet Analytical Instruments in Waltham, MA, USA. Samples of iso-α-acid solution and mixtures of PZ with iso-α-acid at varying molar ratios of 1:0, 1:16, and 1:100 were subjected to freeze-drying to form solid powders for subsequent analysis. To capture the secondary structure of PZ during the foaming process, PZ-iso-α-acid mixtures were stirred to induce foaming, rapidly frozen using liquid nitrogen, and then lyophilized to create solid foam samples for testing. These freeze-dried powders were combined with potassium bromide and scanned over a wave number range of 4000 to 400 cm^{-1}. The procedure involved a total of 32 scans with a spectral resolution of 4 cm^{-1}.

2.6. Foaming Performance

The assessment of foaming performance was conducted based on a slightly adapted procedure from the work of Dai et al. and Chen et al. [17,22]. The preparation of the sample solution involved blending Protein Z (PZ) at a concentration of 0.2 mg/mL with iso-α-acid in varying molar ratios, specifically 1:0, 1:4, 1:16, and 1:100. Within a 50 mL cylinder, 15 mL of this solution was agitated for 90 s at a rotational speed of 9000 rpm using a high-shear mixer device, the Ultra TURRAX homogenizer T25 digital from IKA, Staufen, Germany, to generate foam. The initial volume of the created foam, denoted as V_0 (mL), was documented as a function of time. The calculation of foamability was executed utilizing the following formula (Equation (1)).

$$\text{Foamability } (\%) = 100 \times \frac{V_0}{15} \quad (1)$$

Once the primary foam had formed, it contained residual liquid, causing a swift increase in the liquid level and subsequent compression of the foam volume, which obscured the true measure of foam stability. Consequently, the measurement of foam stability commenced at a point where the liquid level was largely stable, occurring at the 5 min mark.

The volumes of foam at 5 min (V_5) and 30 min (V_{30}) were designated as the representative values for this phase. The subsequent formula (Equation (2)) was then applied to determine the stability of the foam:

$$\text{Foamstability } (\%) = \frac{V_5}{V_{30}} \quad (2)$$

Furthermore, to assess the foam's texture and bubble size distribution, the freshly created foam was transferred onto a microscope slide for examination under an optical microscope, which facilitated the visualization of the foam's fineness.

2.7. Surface Pressure and Surface Tension

Surface tension and surface pressure are closely related and are crucial in enhancing the shaping capabilities of protein solutions [23]. The following formula (Equations (3) and (4)) was then applied to determine the interplay between surface tension (γ) and surface pressure (π):

$$\pi = \gamma_0 - \gamma \quad (3)$$

$$\pi = 2C_0KT(D_t/3.14)^{1/2} \quad (4)$$

In this equation, π (measured in mN/m), γ_0 (also in mN/m), and γ (in mN/m) correspond to the surface pressure of the test solution, the surface tension of the citric acid buffer solution (50 mM, pH 4.4), and the surface tension of the test solution, respectively. The evolution of surface pressure over time is described by Equation (4), which is not provided in your text but would typically involve variables representing the sample concentration, Boltzmann constant, absolute temperature, diffusion coefficient, and the time of adsorption.

2.8. Protein Penetration and Molecular Reorganization

At the end of the short protein diffusion adsorption process, the rate of adsorption gradually decreases, which is due to the fact that it is gradually affected by osmosis and molecular reorganization. The rate of permeation and molecular reorganization of proteins at a given interface can be calculated by Equation (5) [24].

$$\ln\left(\frac{\pi_f - \pi_t}{\pi_f - \pi_0}\right) = -k_i t \quad (5)$$

where π_f represents the final time of adsorption, π_t represents the arbitrary time, π_0 represents the initial time, and ki represents the first-order rate constant.

2.9. Statistical Analysis

All experiments were performed in triplicate. All data are presented as mean ± standard deviation (n = 3). One-way analysis of variance (ANOVA) was utilized for statistical analysis, followed by Duncan at $p < 0.05$.

3. Results

3.1. Preparation of PZ and Iso-α-Acid

Protein Z (PZ), a single-unit protein with an approximate molecular mass of 40 kilodaltons (kDa), was confirmed as effectively purified for subsequent experimental utilization. This conclusion was drawn from Figure S1, where PZ was observed as a solitary band across both (SDS-PAGE) and native polyacrylamide gel electrophoresis (PAGE), with the molecular weight aligning precisely with the 40 kDa. As another part of this study, iso-α-acid are isomers of α acids and are produced by isomerization reactions during the processing and storage of hops. The retention time of iso-α-acid standard was 10.183 min by HPLC liquid chromatography; meanwhile, the retention time of the iso-α-acid sample

was 10.167 min. The retention times after purification were essentially the same as those of the iso-α-acid standards (Figure S2). Further calculation of the peak area shows that the purity of the sample was calculated to be 68.95%. The iso-α-acid prepared by us was used in the following experiments.

3.2. Interaction Between PZ and Iso-α-Acid

3.2.1. Molecular Interactions and Stoichiometric Correspondence Between PZ and Iso-α-Acid

In order to investigate the stoichiometric number relationship between PZ and iso-α-acid, we constructed and analyzed the effect of iso-α-acid on the microenvironment around PZ tryptophan residues. As depicted in Figure 1a,b, the fluorescence intensity of PZ progressively diminished following the introduction of iso-α-acid and, as can be seen from Figure 1c, the molecular particle size of the samples showed a steady increase with the addition of iso-α-acid. This is strong evidence that there is an interaction between them. In order to confirm the binding constant, the following equation was utilized for fitting. In an effort to determine the binding constants for iso-α-acid, a fitting procedure was conducted in accordance with Equation (6).

$$I = I_0 - \frac{I_0 - I_\infty}{2n[P]_0} \times [\frac{1}{K} + [H]_0 + n[P]_0 - \sqrt{\left(\frac{1}{K} + [H]_0 + n[P]_0\right)^2 - 4n[H]_0[P]_0} \quad (6)$$

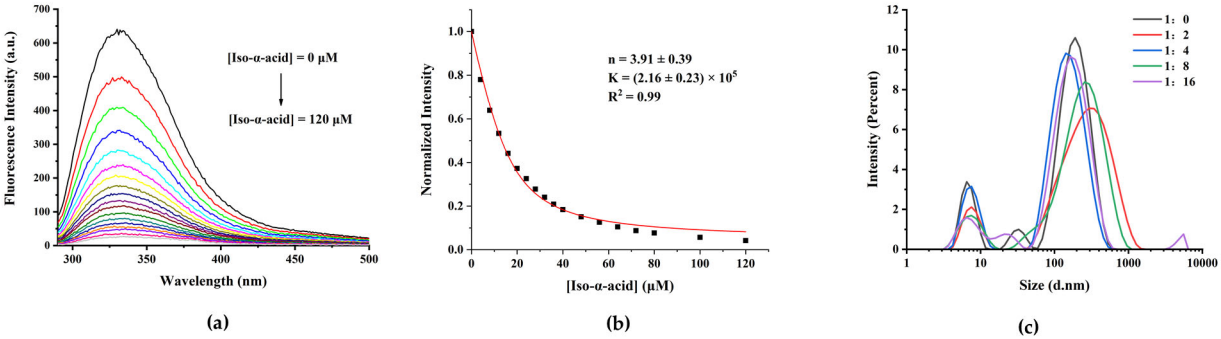


Figure 1. (**a**,**b**) An increase in iso-α-acid concentration leads to fluorescence quenching of the PZ. A curve fitted according to Equation (6) is shown. The color lines arranged from high to low in the figure demonstrate the gradual quenching of the fluorescence with the gradual increase in the addition of iso-α-acid, proving that there is a reaction between the two. (**c**) PZ molecular particle size scale increases with increasing iso-α-acid incorporation.

In this equation, n and K correspond to the binding ratio and the binding coefficient, respectively. I_0 and I_∞ denote the fluorescence intensities of PZ in the absence and presence of saturated iso-α-acid, respectively. $[P]$ and $[H]$ indicate the concentration levels of PZ and iso-α-acid, respectively. From the experimental results and data fitting results, it can be seen that each PZ molecule can bind 3.91 ± 0.39 iso-α-acid molecules with a binding constant K = (2.16 ± 0.23) × 10^5 M^{-1}, relatively better binding capacity compared to other small molecules [25].

3.2.2. Effect of Iso-α-Acid Binding to PZ on Its Structure

In order to analyze the mechanism of interaction between iso-α-acid and PZ at the molecular level, we analyzed the PZ-iso-α-acid complex using circular dichroism and MD simulation. To precisely characterize the interaction between iso-α-acid and PZ on this basis, we performed a residue decomposition of the binding energy, with amino acids such as Leu 396 contributing most to the binding of the complex. Hydrogen bonding played an important role in the binding of iso-α-acid to the PZ during the MD process, with three

hydrogen bonds present throughout the simulation (Figure 2). Specifically, iso-α-acid forms hydrogen bonds with leucine 396, serine 292, and lysine 290, respectively.

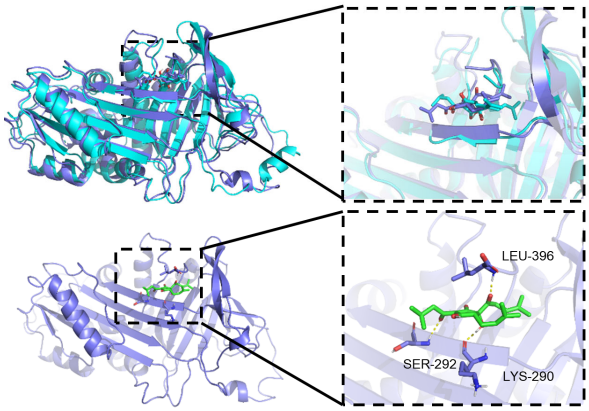


Figure 2. The binding situation of PZ–iso-α-acid after MD. The green molecules represent iso-α-acid molecules, while the blue molecules represent PZ molecules. The yellow dotted line represents the hydrogen bond.

3.3. Influence of Iso-α-Acid on PZ Foaming Properties

A beer's foam is a critical attribute that influences its sensory experience. Numerous factors can impact the beer's foam formation and the longevity of its foam. Initially, an examination was conducted to determine the effects of iso-α-acid on the characteristics of PZ foam. The PZ concentration was maintained at a constant level (0.2 mg/mL), and the foam-forming capacity of PZ solutions with varying molar ratios of iso-α-acid was documented. It was observed that there was a notable increase in the initial volume of foam as the amount of iso-α-acid increased (Figure 3a). Upon statistical analysis, it was found that foamability has a stable and obvious continuous rise with increasing molar ratio ($p < 0.05$). When the molar ratio of PZ to iso-α-acid was 1:20, the foamability was 70.0 ± 3.33% ($p < 0.05$), and when it came to 1:80 and 1:250, the foamability was 97.78 ± 13.88% ($p < 0.05$) and 113.33 ± 6.67% ($p < 0.05$). When the molar ratio reached 1:500, the foamability reached 137.78 ± 7.7% ($p < 0.05$). This was about two times higher than pure PZ (57.78 ± 3.85%) ($p < 0.05$) (Figure 3b).

In order to analyze the interfacial characteristics of PZ-iso-α-acid, referring to the method of Othmeni I et al. [26], the surface tension was measured, which serves as an indicator of protein surface activity. As depicted in Figure 4a, the surface tension for all samples rapidly decreased within a short time and then gradually leveled off to an equilibrium state, indicating continuous adsorption of PZ to the air/liquid interface and a progressive approach to saturation. It is evident that the surface tension at all measured time points was lower after the addition of iso-α-acid compared to pure PZ. Furthermore, as the content of iso-α-acid increased, the surface tension decreased, suggesting that the interaction between iso-α-acid and PZ enhances surface activity. The reduction in surface tension implies an increase in surface activity, making it easier to form foam [8].

During the initial phase of adsorption, a direct proportionality between π and the square root of time $t^{1/2}$ signifies that the adsorption process is primarily governed by protein diffusion, with the slope of this relationship being indicative of the diffusion rate, denoted as K_{diff} (as illustrated in Figure 4b). A reduction in K_{diff} is observed following the introduction of iso-α-acid, which suggests that the movement of PZ to the air/liquid interface might be accelerated upon the addition of iso-α-acid, potentially to a degree that is not captured by the measurement method. The K_{diff} value decreased from 0.9438 ± 0.0179 at PZ:iso-α-acid = 1:0 with increasing addition of iso-α-acid to 0.6392 ± 0.0131 at PZ:iso-α-acid = 1:100. At the commencement of monitoring, a substan-

tial quantity of PZ had already reached the interface, which subsequently decelerated the diffusion rate of the remaining PZ. The initial surface pressure of the PZ-iso-α-acid mixture was found to be lower than that of the PZ-only solution (π_0), which supports the aforementioned hypothesis.

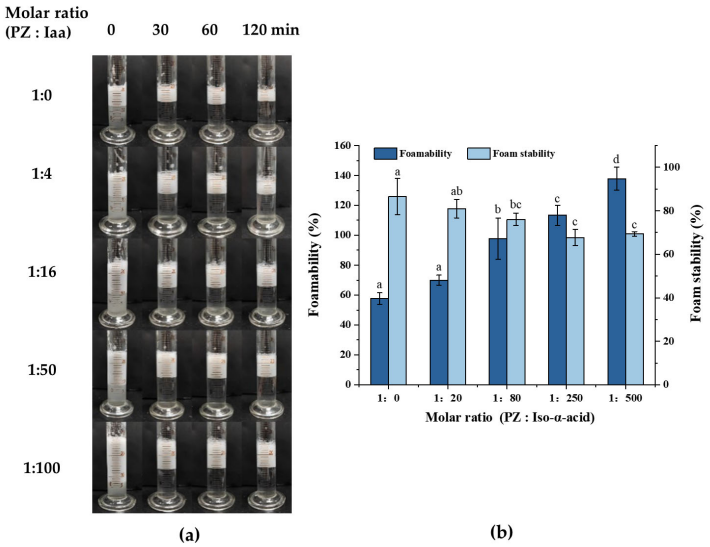


Figure 3. (**a**) The visual foam volume images of PZ-iso-α-acid with different molar ratios after blistering. (**b**) Foamability and foam stability of PZ-iso-α-acid with different molar ratios. Small letters such as a, ab, etc. represent significant differences between groups of data when the significance level is set at 0.05.

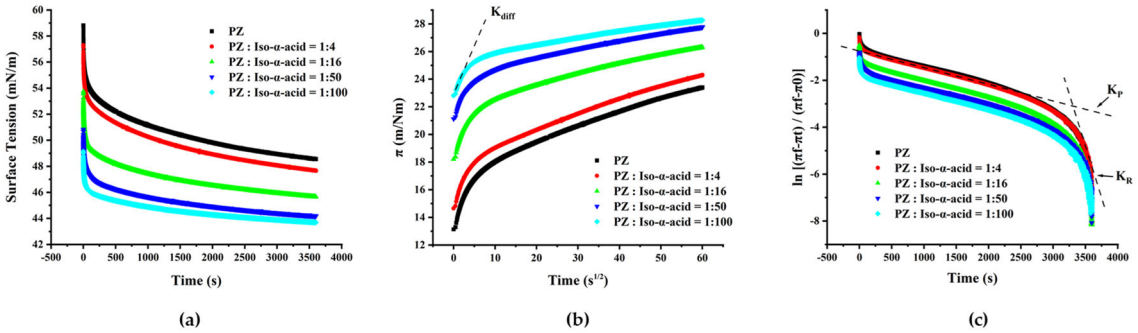


Figure 4. (**a**) The dynamic surface tension of PZ and PZ-iso-α-acid solution at the air/water interface over 3600 s. (**b**) Time dependence of surface pressure (π) for PZ and PZ-iso-α-acid adsorbed films at the air/water interface. (**c**) Typical profile of the molecular penetration and configurational rearrangement steps at the air/water interface for PZ and PZ-iso-α-acid systems.

Two linear phases can be clearly observed from the images. The slope of the first of these phases represents the permeation rate (K_P), while the slope of the second phase represents the molecular reorganization rate (K_R). As shown in Figure 4c, after the addition of iso-α-acid, a gradual decrease in permeability ensues.

3.4. Effect of the Interaction of Iso-α-Acid with PZ on the Stability of Beer Foam

Consumers tend to favor beer with a fine and dense foam. The foam bubbles in beer were examined both visually and microscopically. As depicted in Figure 5, the size of the foam bubbles in the PZ solution with added iso-α-acid is notably smaller than that of the foam from pure PZ, suggesting that the latter's foam is more uniform and delicate.

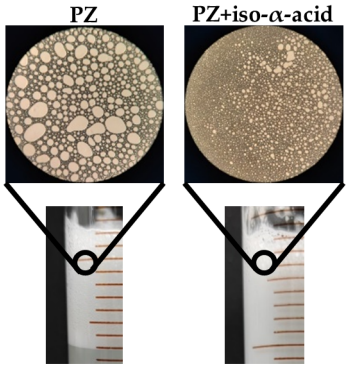


Figure 5. Optical microscope images of the foams of PZ and PZ-iso-α-acid. Scale bar is 1 mm.

The secondary structure of a protein can also indicate the stability of its foam formation to a certain degree. Typically, proteins with a more robust rigid structure exhibit superior foam stability [27]. Proteins that maintain a certain folded structure are more capable of forming thicker films and more stable foams [10,28]. The structure and foam properties of a protein can be altered when it binds to a small molecule due to the interactions between them. Fourier-transform infrared spectroscopy (FTIR) data in the range of 1600–1700 cm^{-1}, which corresponds to the amide I band, were analyzed to determine the secondary structure of PZ before and after the foaming of various samples. Transitioning from solution to foam, there was a decrease in the content of the rigid structure (β-sheet) of PZ and a relative increase in the flexible structure (β-turn). This occurs because the structure partially unfolds after PZ adsorbs at the interface. Furthermore, the addition of iso-α-acid, either in solution or in the foam, resulted in a decrease in the α-helix and β-turn content and an increase in the β-sheet content of PZ. Upon the transition from solution to foam, as in Figure 6a,b, when the value of PZ:iso-α-acid is 1:16, the proportion of β-sheet increased from 23.95% to 26.48%. With the addition of iso-α-acid, when the PZ:iso-α-acid ratio was 1:100, the proportion of β-sheet further rose from 26.63% to 29.74%. Concurrently, the proportions of α-helix and rigid β-sheet structures both decreased to varying extents. This indicates that the binding of iso-α-acid modifies the secondary structure of PZ to some extent, and the increased β-sheet content contributes to the stability of the foam. The hydrogen bonding between PZ and iso-α-acid alters the original intermolecular forces, enhances the adsorption of PZ at the air/liquid interface, and thus enhances its foam-forming ability.

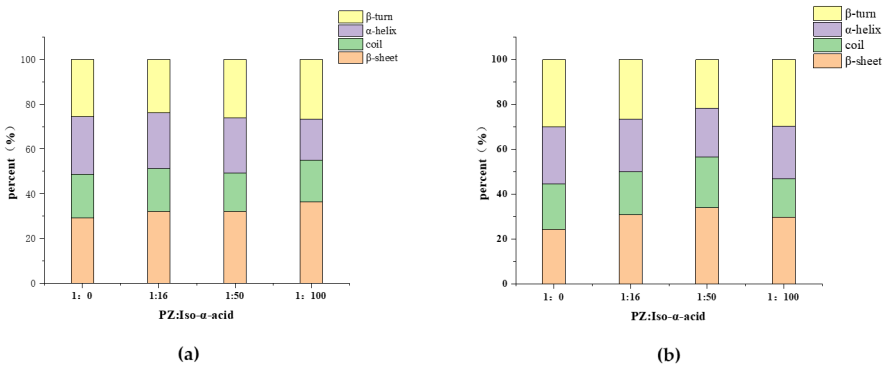


Figure 6. Secondary structural components of PZ-iso-α-acid with different molar ratios in liquid and foam. (**a**) PZ-iso-α-acid secondary structure components in liquids with different molar ratios. (**b**) PZ-iso-α-acid secondary structure components in foams with different molar ratios.

4. Discussion

The quality and stability of beer foam is one of the key factors affecting consumer experience. In this study, it was found that the interaction of iso-α-acid with protein Z could

significantly improve the texture and persistence of beer foam. By adjusting the amount of iso-α-acid added, the characteristics of beer foam can be optimized, thus enhancing the sensory quality of the product. In this study, the interaction between iso-α-acid and protein Z in beer and its effect on the quality and stability of beer foam were thoroughly investigated. Through fluorescence titration experiments and molecular dynamics simulations, we found that there was a significant binding capacity between iso-α-acid and protein Z, with a binding constant of $(2.16 \pm 0.23) \times 10^5$ M^{-1} and a binding ratio of 3.91 ± 0.39. This result suggests that the interaction between iso-α-acid and protein Z by hydrogen bonding to amino acid residues such as Leu-396, Ser-292 and Lys-290. This binding not only changed the secondary structure of protein Z, increasing its β-sheet content from 21.75% to 29.74%, but also significantly enhanced its surface activity and foam stability. These findings provide a new perspective for understanding the physicochemical properties of beer foam and provide a theoretical basis for optimizing the quality of beer foam. Meanwhile, the results of the present study form a useful complement to previous studies. For example, Kunimune et al. [11] investigated the effect of iso-α-acid on the stability of beer foam, but did not delve into its intermolecular interaction mechanism with protein Z. Our study revealed the specific site and mode of action of iso-α-acid binding to protein Z through molecular dynamics simulations, further confirming the importance of this interaction on foam stability. In addition, compared with the study of Xu et al. [14], we not only focused on the effect of iso-α-acid on protein Z structure, but also experimentally verified its significantly enhancing effect on foam formation and stability. These findings provide a more comprehensive guide for optimizing foam quality in beer brewing.

This study focused on the direct interaction between iso-α-acid and protein Z; beer contains other components (e.g., polyphenols, sugars, etc.) which may interact with iso-α-acid or protein Z in a complex manner and thus affect foam characteristics. As mentioned in the study by X Li et al., the glycation of protein Z has been proved to protect it from precipitation in the wort boiling process and improve foam stability [28]. Also noted in the results of Steiner E et al., foam-positive components such as hop acids, proteins, metal ions, gas composition (ratio of nitrogen to carbon dioxide), and gas level, generally improve foam, when increased [29]. Future studies could further explore the synergistic mechanism between these components and their behaviour under different brewing conditions, as well as the interactions between other components of beer with iso-α-acids and protein Z, with a view to providing more comprehensive support for the improvement of beer brewing technology.

5. Conclusions

In this research, we explored how iso-α-acid, a derivative of the hop's α-acid, influences the properties of beer foam, particularly its formation and stability. Utilizing a combination of multi-spectroscopy analysis and molecular dynamics simulation, we discovered that a single molecule of PZ can bind with approximately 3.91 ± 0.39 iso-α-acid molecules, with an affinity constant of $(2.16 \pm 0.23) \times 10^5$ M^{-1}, predominantly through hydrogen bonding. Our findings indicate that the addition of iso-α-acid notably enhances the beer foam's formation, stability, and texture, which are vital for the beer's quality and have not been extensively studied previously. A plausible reason for this improvement is that iso-α-acid forms a complex with the PZ, subtly altering its molecular structure, the percentage of β-sheets increased with iso-α-acid addition from 21.75% to 29.74%, thereby increasing the intermolecular forces, surface tension, and interfacial elasticity. This research lays the groundwork for the advancement of hop cultivation and the enhancement of beer's overall quality.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/chemistry7020065/s1, Figure S1: SDS-PAGE (a) analyses and native PAGE (b) analyses of PZ; Figure S2: HPLC chromatograms of iso-α-acid standard and iso-α-acid sample prepared.

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Data Availability Statement: Data are contained within the article.

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Conflicts of Interest: The authors declare no conflicts of interest.

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