

Improvement of oil-water interfacial behaviors and emulsifying properties of highland barley protein by cold plasma treatment

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ABSTRACT

The focus of this investigation is to explore the effect of cold plasma modification on the stabilization of water-in-oil (W/O) emulsions with highland barley (HB) proteins. It was notable that following cold plasma treatment, the interfacial tension of the HB protein was significantly reduced, and its rearrangement rate increased by 43 %. These changes indicate enhanced interfacial activity of the protein, suggesting improved interaction between the HB protein and the liquid interface. The average droplet size of the emulsion decreased to $2.61 \pm 0.09 \mu\text{m}$ in the T4 group (40 min treatment), suggesting a more uniform dispersion of the oil droplets. Concurrently, the absolute value of the Zeta potential ($28.12 \pm 0.73 \text{ mV}$), the viscosity of the emulsion, and the interfacial protein adsorption rate increased. CLSM images confirm that emulsions stabilized by cold plasma-treated HB proteins exhibit smaller and more uniformly distributed droplets. Further confirming that cold plasma treatment improves the emulsification properties of the HB proteins, resulting in more stable emulsions overall. These findings underscore the notion that cold plasma modification represents a promising approach to enhance emulsion stability and possesses considerable potential to augment the functionality of HB protein in a range of applications.

1. Introduction

Highland barley (HB, *Hordeum vulgare* L. var. nudum hook.F), a special economic crop in the Plateau region, has a high protein content and a balanced amino acid composition [1]. Despite its nutritional potential, HB remains underutilised in high-value food applications. This is largely attributed to HB proteins are categorized into four fractions: albumin (12.95 %), globulin (12.73 %), gliadin (16.96 %), and glutenin (47.83 %), with the latter's dominance (glutenin) contributing to poor solubility and limited emulsification capacity [2,3]. Although HB proteins exhibit inherent amphiphilicity, their intricate structural configurations, characterized by substantial molecular aggregation and limited surface hydrophobicity, impede efficient adsorption kinetics at oil-water interfaces, thereby compromising emulsion stability through accelerated droplet coalescence [4–6].

To overcome these limitations, protein modification strategies such as enzymatic hydrolysis, chemical modification and ultra-high-pressure

homogenization have been explored but face challenges of high cost and energy consumption [7]. In recent years, cold plasma technology offers a sustainable alternative due to its unique advantages: low operating temperature, no need for external chemical reagents, cost-effectiveness, and the ability to improve protein functionality while preserving its nutritional value [8]. Cold plasma is produced by ionization of neutral gases in response to an energy input [9]. The reactive oxygen and nitrogen species generated by cold plasma have the potential to interact with electron-rich groups in amino acids, thereby influencing the hydrophilicity of these amino acids [10]. Notably, cold plasma has proven effective in modifying plant protein functionality [11–13]. For example, Zhang et al. [14] reported a 282 % increase in SPI solubility after 3 min treatment. Corn protein isolate (CPI) and wheat germ proteins treated with cold plasma exhibit enhanced emulsification capabilities, which are contingent upon the treatment duration and pH level (J. [15,16]). While previous studies on cold plasma modification of plant proteins have focused on solubility or emulsification capacity at fixed treatment

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durations, the relationship between plasma exposure time and interfacial behavior (e.g., adsorption kinetics, interfacial film elasticity) remains unexplored.

Our prior work [17] confirmed cold plasma induced secondary structural changes in HB protein, including glutenin aggregate dissociation and reduced α -helix content. However, the structural modifications driving interfacial adsorption and emulsion stabilization remained unclear. This study systematically investigates how prolonged plasma treatment (0–40 min) modulates HB protein's structural flexibility and interfacial adsorption efficiency, providing quantitative correlations between treatment duration and emulsion stability parameters (e.g., droplet size, ζ -potential, viscosity). By correlating treatment duration with structural, interfacial, and emulsification metrics, this work provides mechanistic insights into HB protein modification and advances its application in plant-based emulsified products.

2. Materials and methods

2.1. Materials

Highland barley (HB) (XiNing KunLun 18) was provided by the QingHai Academy of Agriculture and Forestry Sciences. Sunflower oil was purchased from standard foods (China) Co., Ltd. PBS (phosphate buffer saline) was obtained from Beijing Chemical Industry Group Co., Ltd. (Beijing, China). Nile Red and Nile Blue were purchased from Solarbio Science & Technology Co., Ltd. (Beijing, China). All other chemicals used were of analytical grade and were provided by Sino-pharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. HB protein preparation

Alkaline extraction followed by isoelectric precipitation was employed to prepare HB protein according to the method reported by Du et al. [18] with some modifications. In brief, HB grains were milled using a rice mill (RSKM 3D, SATAKE MultiMix Co., Ltd., Japan) to remove the bran, and then soaked overnight in deionized water at a ratio of 1:15 (w/v). The soaked mixture was blended for 1 min, 4 times using a high-speed blender (L12-Energy61, Joyoung Company Limited Co., Ltd., China). The homogenate was adjusted to pH 10.5 with 2 M NaOH, and centrifuged at 4500 rpm for 10 min. The pH of supernatant was adjusted to 4.5 with 2 M HCl, the precipitate was washed and freeze-dried (using a freeze-dryer DG-65Z04-10 A, QingDao Haier Biomedical Co., Ltd., China). The protein content in the HB protein was determined to be $84.5 \pm 0.5\%$ using the Kjeldahl method with a protein conversion factor of 5.83 [19]. The HB protein is stored at -20°C refrigerator standby.

2.3. Cold plasma treatment

HB protein powder (2 g) was evenly distributed in a 90 mm diameter petri dish. Cold plasma treatment (Institute of Apicultural Research, Chinese Academy of Agricultural Sciences) was performed under the following conditions: voltage 30 kV, frequency 50 Hz, electrode gap distance 4 cm, using air as the process gas and reactive species source. Upon application of the appropriate voltage, the air within the reactor was ionized, generating oxygen-containing compounds. Samples were treated for 0, 10, 20, 30, and 40 min, collected and stored at -20°C . These were designated as T0, T1, T2, T3, and T4, respectively.

2.4. HB protein dynamic interfacial pressure

The dynamic interfacial pressure of HB protein adsorption at the oil-water interface was measured using an OCA20 video optical contact angle measurement instrument (Bruker alicona Co., Ltd., China). Before the experiment, the system was rinsed multiple times with the test solution using a syringe. Using the instrument's auto-injection function, 16

μL of HB protein solution was filled into a glass test tube containing about 3/4 of the volume of sunflower oil (density 0.92 g/cm^3).

The change in interfacial tension was monitored by capturing images of the sample droplet. Light and external vibrations should be avoided during the measurement process. The dynamic interfacial pressure (π) was calculated using the following equation through Eq. (1) (J.-M. [20]):

$$\pi = \lambda_0 - \lambda_t \quad (1)$$

where λ_0 (mN/m) represents the interfacial tension of PBS buffer in sunflower oil ($21.51 \pm 0.14\text{ mN/m}$). λ_t represents the real-time interface tension.

The interface tension curve as a function of time was obtained to evaluate adsorption mechanism and interfacial behavior of HB protein on the oil-water interface according to the asymptotic Ward and Tordai equation through Eq. (2) [21].

$$\ln \left[\frac{\lambda_f - \lambda_t}{\lambda_f - \lambda_0} \right] = -K_i t \quad (2)$$

where λ_f is the final interface tension, λ_0 is the initial interface tension, λ_t is the real-time interface tension, K_i is the first-order rate constant and t is the testing time.

2.5. HB protein emulsion preparation

The emulsion was prepared with reference to the method of Zhang (B. [22]). The HB protein was prepared into 2 % (w/w) suspension with 10 mM pH 8.0 PBS at different treatment times (0, 10, 20, 30, 40 min). The suspension was stirred at room temperature for 5 h and adjusted the pH to 7.0 using 1 M HCl, then stirring overnight to fully hydrate. The sunflower oil was mixed with the supernatant at a ratio of 12:1 (w/w), and the mixture was dispersed in a high-speed disperser (XHF-D, NingBo Scientz Biotechnology Co., Ltd., China) at 10,000 rpm for 2 min. The dispersion was homogenized at 100 Pa and then homogenized 3 times at 800 Pa using a homogenizer (HPH-L2, Aitesen Co., Ltd., China) to obtain HB protein emulsion.

2.6. Adsorption rate of interfacial protein

The freshly prepared HB emulsion (2 mL) was centrifuged at 10,000g and 4°C for 20 min. The serum (transparent) phase was filtered through a $0.45\text{ }\mu\text{m}$ filter membrane using a disposable syringe to obtain the filtrate for protein concentration determination. The protein concentration in the filtrate was measured using a Bradford kit (Beijing Boxbio Science&Technology Co.,Ltd). The ratio of adsorbed protein (AP, %) was calculated as follows through Eq. (3):

$$\text{AP\%} = (C_0 - C_1)/C_0 \quad (3)$$

where C_0 (mg/mL) represents the original concentration of the protein solution before emulsification, and C_1 (mg/mL) represents the protein concentration in the filtrate.

2.7. Droplet size distribution of the emulsion

The droplet size distribution of the HB emulsion was determined using a laser droplet size analyzer (Sync Microtrac Co., Ltd., USA) and reported as the volume-weighted mean diameter ($d_{4,3}$) [23]. An appropriate volume of the emulsion was extracted and placed in the sample cell of the analyzer. The refractive indices of the emulsion droplets and the dispersing medium were set to 1.56 and 1.33, respectively.

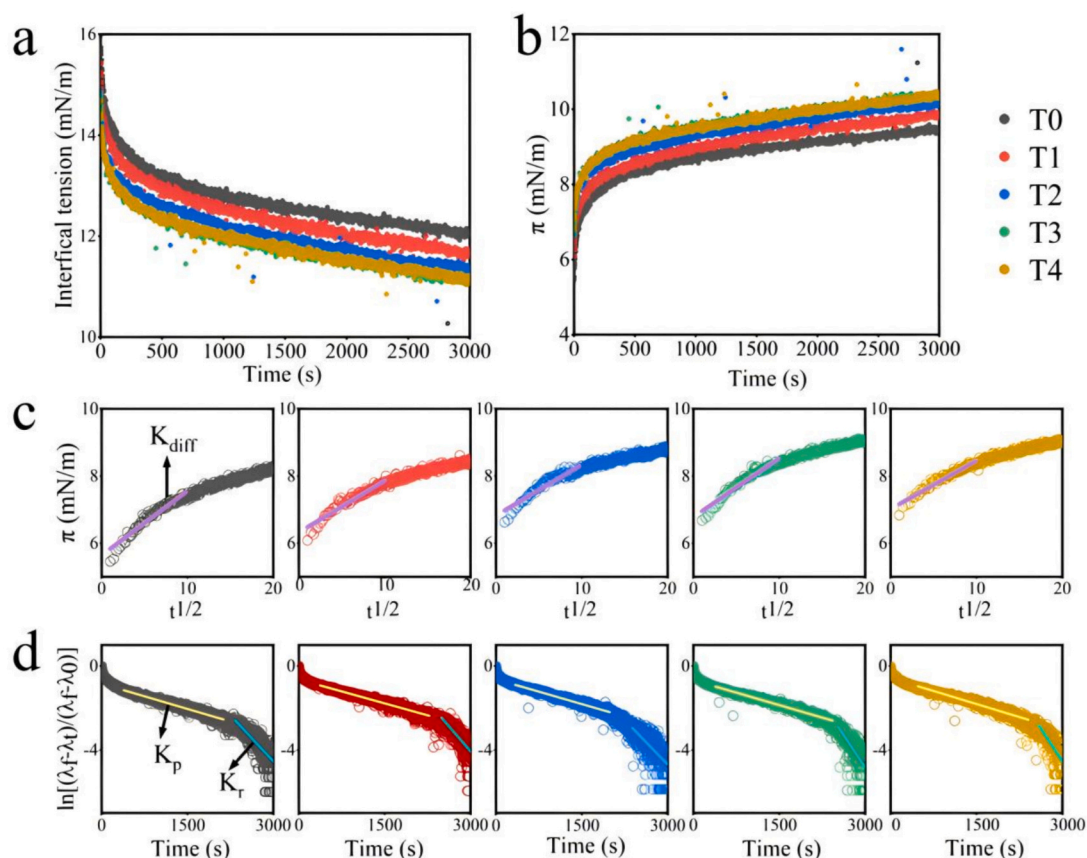


Fig. 1. Effects of cold plasma treatment on dynamic interfacial pressure of HB protein. Time evolution of interfacial tension (a), time evolution of surface pressure (b), the square root of time ($t^{1/2}$) dependence of interfacial pressure (π , mN/m) (c), time dependence of $\ln[(\pi_{3000}-\pi_t)/(\pi_{3000}-\pi_0)]$ (d).

2.8. ζ -Potential of the emulsion

The ζ -potential of the emulsion was measured using a Malvern zeta potential analyzer (Zetasizer Nano-ZS90, Malvern Co., Ltd., UK), as described by Hu and Zhang [24]. The emulsion sample was diluted to a ratio of 1:100, with 0.01 M pH 7.0 PBS buffer, after which 1 mL of the diluted emulsion was loaded into the sample cell for measurement.

2.9. Rheological properties of the emulsion

The apparent viscosity of the emulsion was measured using a rotational rheometer (Physica MCR301, Anton Paar Co., Ltd., Austria), which consists of two parallel plates with a gap of 0.047 mm at 25 °C [L. [25]]. The viscosity changes were performed a frequency of 1 Hz in a shear rate of 0.1–100 s⁻¹ for 60 s.

The dynamic viscoelasticity of the emulsion was also measured using the rotational rheometer. The changes in storage modulus (G') and loss modulus (G'') were obtained through scanning frequency range from 1 to 100 Hz, at a 1 % constant strain.

2.10. Confocal laser scanning microscopy (CLSM) of the emulsion

CLSM (LSM T-PMT, Carl Zeiss AG Co., Ltd., Germany) equipped with a 20 $\times$ objective lens [26]. A mixture comprising 20 μ L of 0.1 % Nile Red dye and 1.0 % Nile Blue dye was added to 1 mL of the emulsion and mixed well. Nile Blue was used to label proteins, while Nile Red was used to label the internal oil phase. The stained emulsion was diluted 5 times with pH 7.0 buffer before dropped onto a glass slide, and two laser excitation sources (488 nm and 633 nm) were used to analyze Nile Blue and Nile Red, respectively. The entire process was light-protected prior to analysis.

2.11. Turbiscan stability analysis (TSI) of the emulsion

Turbiscan stability analyzer (TLAB AGS, Fomulaction Co., Ltd., France) was employed to study the stability of the samples in accordance with the methodology proposed by Shen [27]. The freshly prepared HB emulsion (20 mL) was placed in a cylindrical glass cell, then scanned every 30 min for 24 h at 25 °C.

2.12. Statistical analysis

The experimental data was subjected to the variance analysis (ANOVA) with SPSS Statistics 19.0 (SPSS Inc., Chicago, IL) and plotted using Origin 2021. Significant differences ($P < 0.05$) were determined by Duncan's multiple range test.

3. Results and discussion

3.1. Effect of cold plasma treatment on the dynamic surface pressure of HB protein

Fig. 1a shows that the interfacial tension decreases rapidly followed by a slow change with time, which indicates that the barley protein first diffuses rapidly to the oil-water interface and undergoes slow rearrangement after adsorption. Moreover, the interfacial tension of T4 group was significantly reduced after 3000 s, which indicated that the cold plasma treatment improved the interfacial activity of barley proteins. This may be due to the fact that the reactive free radicals generated during plasma treatment oxidizing proteins, dissociate dissociating protein aggregates or subunit structures, and the hydrophobic groups buried in the interior are more easily exposed thus enhancing the hydrophobicity surface of the protein surface (Y. [28,29]). In addition, the

Table 1

Effect of different treatment times on characteristic parameters, including the diffusion rate (K_{diff}), constants of penetration and structural rearrangement at the oil-water interface (K_p , K_r). Different lowercase letters (a–d) indicate significant differences ($P < 0.05$) in the mean values within the same parameter group.

Treatment time	$K_{diff}(\text{mNm}^{-1} \text{s}^{-0.5})$	$K_p \times 10^4(\text{s}^{-1})$	$K_r \times 10^4(\text{s}^{-1})$
T0	0.1911 ± 0.00542^c	8.0034 ± 0.0376^a	29.3 ± 0.8388^c
T1	0.1539 ± 0.0044^a	7.5874 ± 0.0379^b	31.5 ± 1.1312^{bc}
T2	0.1516 ± 0.00577^a	7.9000 ± 0.0413^c	32.5 ± 2.0432^b
T3	0.1755 ± 0.00504^b	7.9225 ± 0.0339^c	41.3 ± 1.7132^b
T4	0.1447 ± 0.00356^a	8.2603 ± 0.0465^d	41.9 ± 2.1775^a

π of the protein solution increases with the extension of the plasma treatment time (Fig. 1b). T4 shows the highest the surface pressure (π) value, it suggests that more proteins are adsorbed on the interface after a longer period of plasma treatment [30].

To discern the adsorption behavior of HB proteins at different stages, we calculated π and performed quantitative adsorption kinetic analysis. Fig. 1c shows the square root of π of HB protein as a function of time ($t^{1/2}$). If the curve of π versus $t^{1/2}$ is linear, it indicates that the adsorption process is mainly controlled by diffusion, and the slope of the curve reflects the diffusion rate (K_{diff}). For adsorption kinetics controlled by penetration and rearrangement, analysis of the $\ln[(\pi_t - \pi_\infty)/(\pi_0 - \pi_\infty)]$ - t curve reveals two linear regions, with K_p representing the penetration rate and K_r representing the rearrangement rate (Fig. 1d).

Table 1 shows K_{diff} is lower in all groups treated with cold plasma than in the control. This result can be attributed to the process of diffusion, dissociated HB proteins aggregate due to intermolecular interaction forces, resulting in the formation of larger aggregates [13]. Notably, in the T4 group, the rearrangement rate (K_r) increased by 43 % compared to the control (Table 1). This enhancement is likely

attributable to plasma-induced structural loosening, characterized by reduced α -helix content and disrupted hydrogen bonds [17]. These changes enhance molecular flexibility, allowing proteins to adopt a partially disordered conformation with weakened structural order [31]. Such flexibility facilitates efficient rearrangement at the oil-water interface, ultimately forming a tightly packed and stable protein membrane. [32,33]. This finding is consistent with a previous study by J Li et al. [34] who found that cold plasma modified whey proteins have a greater ability to reduce interfacial tension compared to natural whey proteins.

3.2. Effect of cold plasma treatment on interface protein content of HB protein emulsion

The adsorption rate of proteins refers to the percentage of protein content adsorbed on the surface of oil droplets compared to the protein content in the continuous phase. It can affect the aggregation and flocculation stability of the emulsion. When the interface is fully covered, it maintains the stability of the emulsion and prevents the coalescence and flocculation of the oil droplets. Fig. 2 shows the changes in interface protein content in different groups of HB protein emulsion with cold plasma treatment time. The protein adsorption rate in the control group was the lowest at 59.44 ± 3.66 %, whereas the T1 and T2 groups had protein adsorption rates of 63.35 ± 4.78 % and 69.33 ± 4.84 %, respectively. The protein adsorption rate of T3 and T4 groups still increased slightly with the increase of treatment time. This result corresponds to the experimental results of interface pressure (Fig. 1b), where a higher interface protein content corresponds to a larger π . The etching effect of the cold plasma gives rise to the formation of numerous irregular structures on the protein surface, thereby inducing the fragmentation of aggregates [35]. Loosely structured HB proteins exhibit a propensity for multilayer adsorption at the oil-water interface, resulting

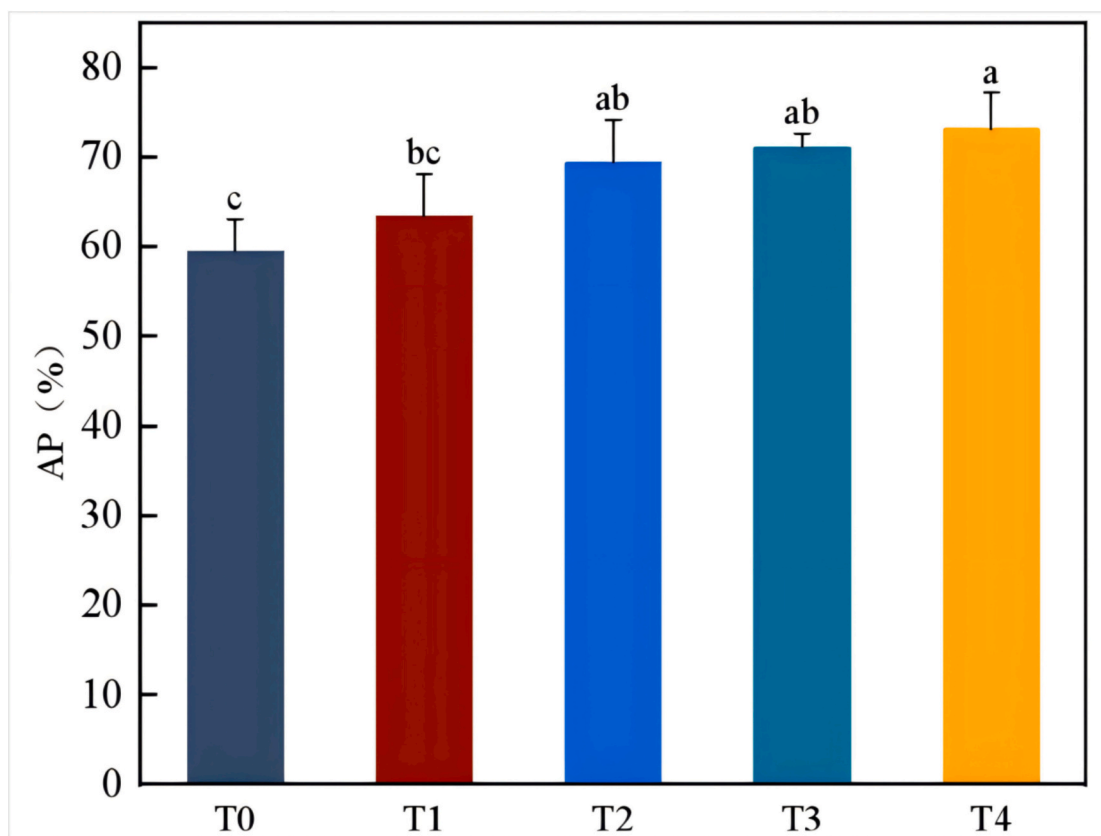


Fig. 2. Effect of cold plasma treatment on the adsorption rate of HB protein. Different lowercase letters (a–c) indicate significant differences ($P < 0.05$) in the mean values within the same parameter group.

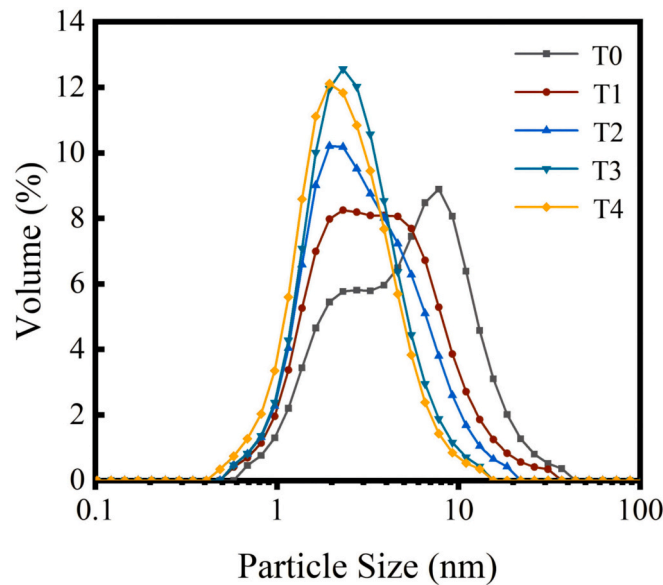


Fig. 3. Effects of cold plasma treatment on the droplet size distribution of HB protein emulsion.

Table 2

The volume-weighted mean diameter ($D_{4,3}$) and ζ -potential values of HB protein emulsion at different treatment times. Different lowercase letters (a–e) indicate significant differences ($P < 0.05$) in the mean values within the same parameter group.

Treatment times	$D_{4,3}(\mu\text{m})$	Span	ζ -potential(mV)
T0	6.09 ± 0.01^a	2.43 ± 0.44^a	-21.77 ± 0.14^a
T1	4.13 ± 0.12^b	2.41 ± 0.11^a	-23.42 ± 0.40^b
T2	3.36 ± 0.05^c	2.13 ± 0.02^a	-24.97 ± 0.05^c
T3	2.75 ± 0.04^d	1.62 ± 0.02^b	-27.37 ± 0.38^d
T4	2.61 ± 0.09^e	1.71 ± 0.05^b	-28.12 ± 0.73^d

in the formation of a more substantial interfacial film. Additionally, the increase in interface protein content may be related to the increase in protein solubility [36]. This aligns with the findings of Deng et al. [37], where appropriate cold plasma treatment increased the solubility of walnut protein isolate by 2.28 times while improving emulsifying stability. And the effect of protein solubility on the interface protein content of heat-treated HB protein isolate emulsions has been reported previously [38].

3.3. Effect of cold plasma treatment on the droplet size distribution and ζ -potential of HB protein emulsion

The droplet size distribution graphs of emulsions prepared with different treatment times for barley protein are shown in Fig. 3. The distribution curve of the emulsion treated showed a narrower and steeper peak with plasma treatment, indicating a more uniform droplet size distribution of the emulsion. Furthermore, the volume-weighted mean diameter ($D_{4,3}$) was used to evaluate the droplet size of HB protein emulsions at different cold plasma treatment times (Table 2). The $D_{4,3}$ decreased significantly with the increase of treatment time ($P < 0.05$), noteworthy at T4 group yielding the smallest $D_{4,3}$ (2.61 μm). It may be due to the increase of AP with increasing treatment time (Fig. 2), resulting in the formation of a thicker and more elastic interfacial membrane around the oil droplets, which protects oil droplets from aggregation and reduces droplet size [39]. When Span approaches zero, it signifies an enhancement in the homogeneity of the particle size, thereby accentuating the consistency of the particle size (Table 2). These results indicate that cryogenic plasma-modified protein emulsions exhibit a reduced droplet size and a more uniform droplet size distribution as the treatment time increases. Similarly, Mehr et al. (2020) found that treated fenugreek protein exhibited excellent absorption around oil droplets after reacting for 60 s at 18.6 kV, resulting in emulsions with minimal droplet size distribution and higher emulsion stability.

The ζ -potential reflects the mutual repulsion or attraction between droplets, and a higher absolute value of the ζ -potential indicates greater stability of the overall system [40]. The ζ -potential values of HB protein emulsion prepared under different treatment times are shown in Table 2. Notably, the absolute value of the ζ -potential of the HB protein emulsion significantly increased following cold plasma treatment (rising from 21.77 mV to 28.12 mV). This may be related to the electrostatic interactions among protein molecules, the elevated surface charge during treatment increases the net charge on the protein surface, which in turn elevates the net charge on the emulsion droplet surface. Such an increase can lead to enhanced intermolecular electrostatic repulsion between emulsion droplets, thereby improving the emulsion stability (C.-B. [41]). However, prolonged processing times can result in overcrowding of the protein micelles and a reduction in the active site of the reaction, ultimately resulting in a decrease in zeta-potential (P. [42]).

3.4. Effect of cold plasma treatment on rheological properties of HB protein emulsion

The rheological properties of an emulsion are important indicators of its performance, which are influenced by the protein structure and

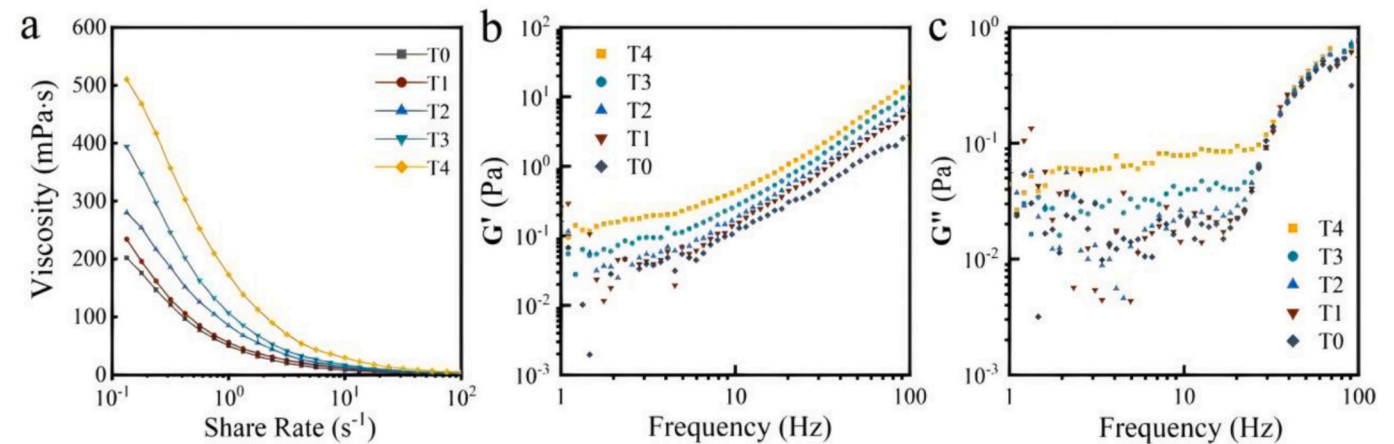


Fig. 4. Effects of cold plasma treatment on the rheological properties of HB protein emulsion. Viscosity changing with the shear rate (a), rheological behavior of storage modulus (G') (b) and loss modulus (G'') (c).

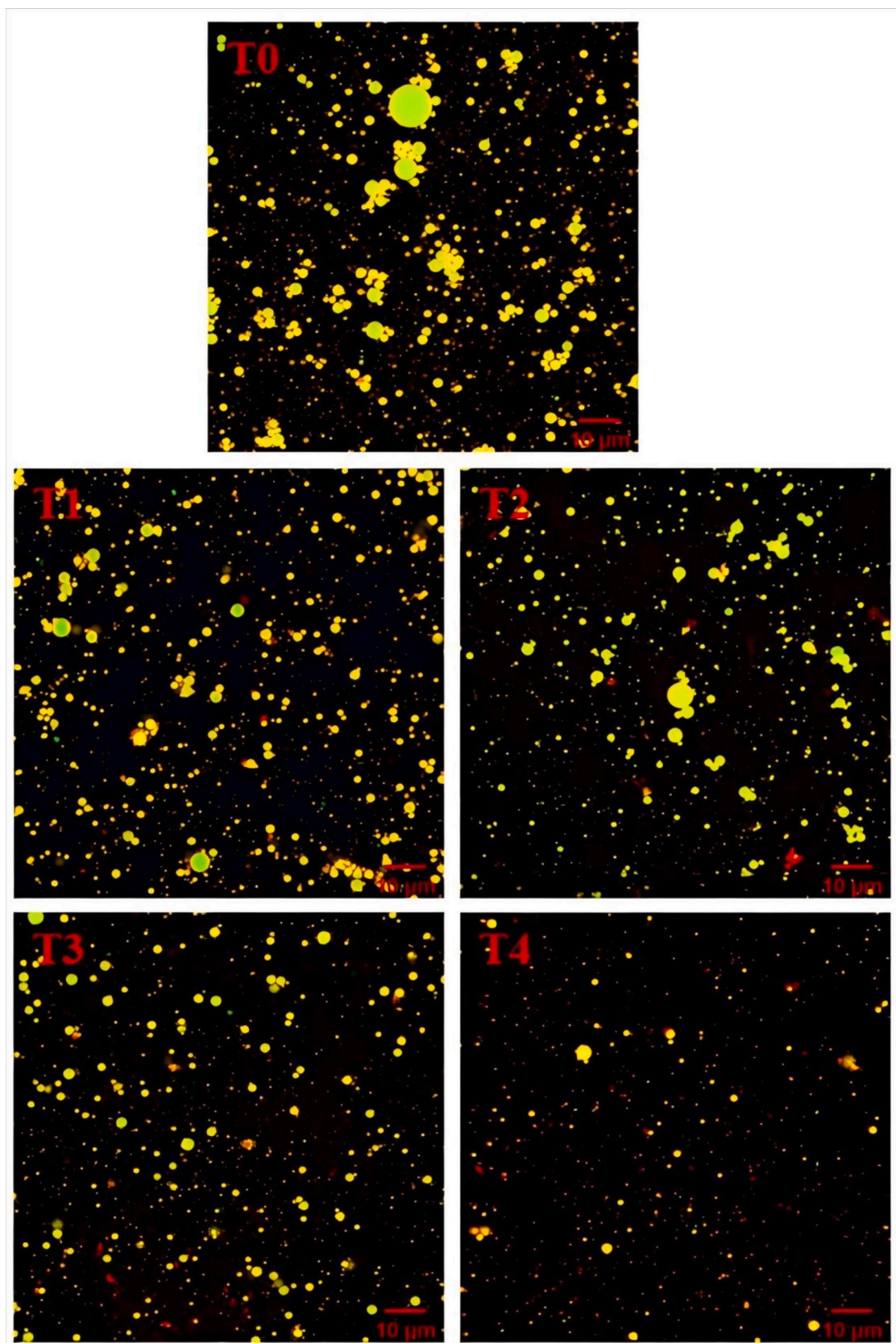


Fig. 5. Effects of cold plasma treatment on the microstructure of HB protein emulsion.

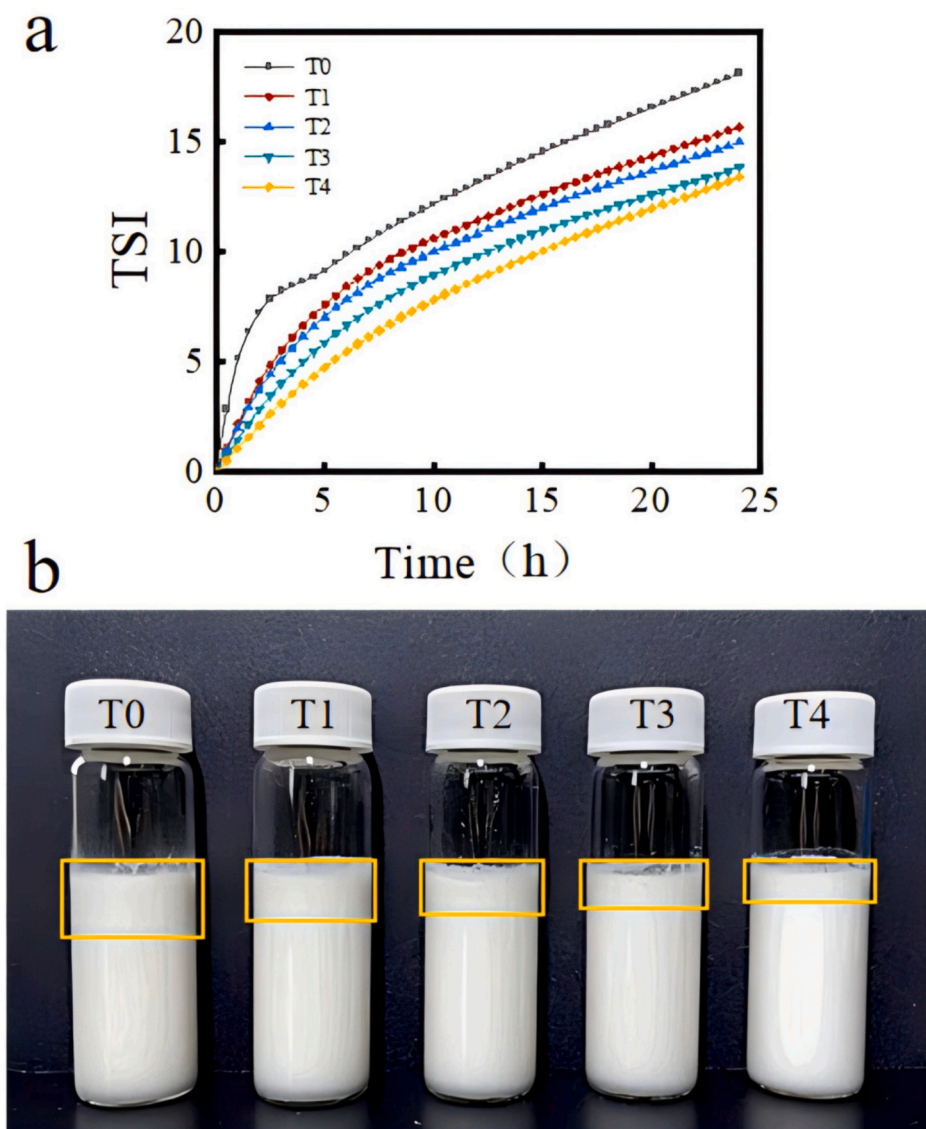


Fig. 6. Effects of pH-shifting treatments on the Turbiscan stability index (a), the creaming state of emulsions after storage for 24 h(b).

interdroplet interactions [43]. As shown in Fig. 4a, all samples exhibited shear thinning within the shear rate range of 0.1 to 100 s^{-1} . As the shear rate increased, the emulsion polymer network disassembled, and macromolecules rearranged in the shear direction, resulting in a decrease in apparent viscosity of the emulsion (Q. [44]). The stabilized emulsion showed significantly higher viscosity than the control group, and the apparent viscosity of the emulsion gradually increased with prolonged cold plasma treatment time, reaching the maximum in the T4 group. This may be attributed to the reduction in emulsion droplet size and the formation of more uniform oil droplets after plasma treatment, leading to increased resistance during shear. According to Stokes' law, the increase in emulsion viscosity is beneficial for slowing down the floating speed of droplets and maintaining better stability [45]. Similar outcomes were observed by Chen et al. [28] when studying on coconut protein emulsion after cold plasma modification at 60 kV for 60 s and 90 s.

Fig. 4b-c illustrates G' and G'' of HB protein emulsions by measuring the dynamic viscoelasticity. It can be observed that G' is significantly higher than G'' across angular frequency sweep range of 0 – 100 rad/s , indicating that the modified HB protein emulsion primarily exhibits elasticity and establishes a three-dimensional network structure. The G' for T1–4 was higher than that of T0. Emulsions exhibited higher

elasticity. As reported by Tang [46], if more proteins are adsorbed at the oil-water interface, the emulsions are more likely to bridge together to form a gel network structure.

3.5. Effect of cold plasma treatment on microstructure of HB protein emulsion

Confocal laser scanning microscopy (CLSM) is commonly used for the analysis of the microstructure of emulsions, providing insights into droplet size distribution, dispersion, and stability (Y.-Q. [47]). The reduced droplet size and uniform distribution observed in plasma-treated emulsions, as shown in Fig. 5, align with findings from studies on other plant proteins. For instance, Ji et al. [48] reported that cold plasma-treated peanut protein formed smaller and more homogeneous droplets in Pickering emulsions, attributed to reduced protein aggregation. Similarly, J. Wang et al. [16] demonstrated that atmospheric plasma treatment of chickpea protein led to a 35 % reduction in droplet diameter, driven by enhanced protein flexibility and interfacial adsorption. In our study, the T4 group achieved a remarkable droplet size reduction to $2.61\text{ }\mu\text{m}$ (Table 2), surpassing the performance of many plant protein-based emulsions (e.g., $3.8\text{ }\mu\text{m}$ for pea protein; Shen et al., 2022). This improvement may stem from the unique composition of HB

proteins, where plasma-induced dissociation of glutenin aggregates and moderate increase in hydrophobicity [17] facilitates rapid adsorption at the oil-water interface.

3.6. Effect of cold plasma treatment on storage stability of HB protein emulsion

TSI is used to quantify the destabilization process of a mixture, where a higher TSI value indicates poorer stability [49]. As shown in Fig. 6a, during the 24-h storage period, the TSI curves of all treatment groups increased with longer storage time. Additionally, the T0 group had the highest TSI value compared to the other groups, indicating the poorest stability. The TSI curves of the emulsions in each treatment group were lower than that of the control group. Visual stability observations were consistent with the TSI curves (Fig. 6b), where fluctuations in the bottom and top layers were more pronounced in the control sample, while smaller in the modified samples. The enhanced storage stability of plasma-treated emulsions can be mechanistically linked to two key factors: (1) the formation of a viscoelastic interfacial film and (2) elevated electrostatic repulsion [50]. The increased G' values (Fig. 4b) indicate a stronger elastic network, which physically restricts droplet coalescence—a phenomenon also observed in plasma-treated coconut protein emulsions (Y. [28]). Our study extends these observations to HB proteins, demonstrating that prolonged plasma treatment (40 min) achieves comparable stability improvements without requiring additional stabilizers—a critical advantage for clean-label food applications.

4. Conclusions

Studies have shown that cold plasma treatment can significantly improve the emulsifying properties of HB protein, thereby producing more stable oil-in-water (O/W) emulsions. Experiments revealed that the modified HB protein exhibits a significantly enhanced ability to reduce the interfacial tension between oil and water. Prolonging the treatment time results in a significant reduction in the average droplet size of the emulsion, while simultaneously increasing the absolute value of the zeta potential, emulsion viscosity, and protein content adsorbed at the interface. Confocal laser scanning microscopy observations revealed that the treated emulsion exhibited more uniform oil droplet distribution and smaller droplet sizes, consistent with the aforementioned changes in physicochemical properties. Collectively, these effects synergistically enhance emulsion stability. These findings provide positive support for the understanding of plasma modification as an effective technological tool for enhancing protein emulsification and improving protein interfacial behavior, and point the way to the application of this technology for improving the stability of emulsion systems in the food industry. Considering energy consumption considerations, future research could explore combined pH-plasma technologies to shorten processing time, or alternatively address inhomogeneity during powder treatment by treating protein solutions instead.

CRediT authorship contribution statement

Jingjing Yang: Writing – original draft, Software, Methodology, Data curation. **Jiayu Wen:** Writing – original draft, Methodology. **Yonghui Liu:** Writing – original draft, Methodology, Data curation. **Xueqing Wang:** Software, Formal analysis. **Lang Jia:** Validation, Software. **JingFeng Li:** Software, Formal analysis. **Bei Fan:** Resources, Investigation. **LiTao Tong:** Validation, Resources. **Fengzhong Wang:** Writing – review & editing, Supervision. **Lili Wang:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors state that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijbiomac.2025.145708>.

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