STRUCTURAL AND FUNCTIONAL MODIFICATION OF WPI USING VACUUM COLD PLASMA

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Abstract

Cold plasma technology is one type of non-thermal technique which generates several reactive species like such as O, OH, and three atoms or molecules in a metastable state, which are generated by exposing a gas or mixture of gases to an electric field and leading collisions. In this study, we evaluated the impact of cold plasma on the structural and functional properties of WPI. Whey Protein Isolate was treated with cold plasma, applying three different gases, atmospheric (1), argon-atmosphere combination (2), and sulfur hexafluoride (SF6) (3).The process was accomplished under controlled conditions (Frequency and time). The physicochemical and interfacial properties of the samples were evaluated using different methods. Results showed a significant difference ($P<0.05$) in the dilatational surface properties, zeta potential, particle size, the adsorbed layer thickness, and fluorescence intensity of the plasma-treated samples and the non-treated WPI sample(N). A decrease in sulfur content and an increase in detected carbonyl group in the plasma-treated samples represent oxidation reactions utilizing cold plasma that may change the surface properties of the protein.

Materials and method

- **Cold plasma treatments:** Vacuumed cold plasma with different gas, Atmosphere, combination of 50%Argon and 50%Atmosphere and SF_6 gas in Frequency of 13.5 Hz and 60 W power was applied
- **Protein carbonyls and sulfhydryl group measurement:** The amount of carbonyl groups was measured using the method of Segat et al. (2015), using 2,4 dinitrophenyl hydrazine as a reagent. The number of free sulfhydryl groups was determined according to Segat et al. (2015) method using Ellman's reagent.
- **Dynamic light scattering, Zeta potential using (Zeta sizer-Malvern).**
- **Foaming properties (** Waniska and Kinsella (1979))
- **Intrinsic und Extrinsic Surface hydrophobicity (Ricky S.H. Lam, 2015):** Hydrophobicity for each protein solution was measured by both extrinsic fluorescence, using a fluorescent probe method, and intrinsic fluorescence, as a function of emission wavelength.
- **Dynamic surface pressure(Lazidis et al.2016) and Interfacial properties**: Dynamic surface pressure was measured by PAT method (Sinterface Technologie- Berlin)
- **Ellipsometry (Georgi et al.2019).**

Results

The results of pH, polydispersity index (PDI), mean size, and zeta potential of WPI non-treated(N), treated with atmosphere gas(1), combination gas argon and atmosphere (2), SF6(3) solution, showed significant differences between treated and non-treated WPI. The lowest pH belongs to the sample treated with SF6 gas making the biggest size (more aggregation) of protein and the lowest zeta potential values.

Treatment	Size (nm)	PDI	pH	Zeta potential
N	229.53[b]	0.31[a]±0.015	6.17[a]	-27.9±2.36[a]
1	299.86[b]	0.41[b]±0.123	6.15[a]	-29.46±1.18[b]
2	388.76[c]	0.45[b]±0.16	6.01[b]	-26.7±1.36[c]
3	2351[d]	0.8[c]±0.2	3.52[c]	12.96±0.4[d]

Table1 : particle size(nm), PDI, pH, Zeta potential(mv) of WPI

Results

Drainage values results showed sample 2 (a combination of atmosphere gas and argon) had a slight decrease in the drainage values that could be associated with higher foam stability. In terms of foamability, cold plasma decreases slightly the foam volume of protein.

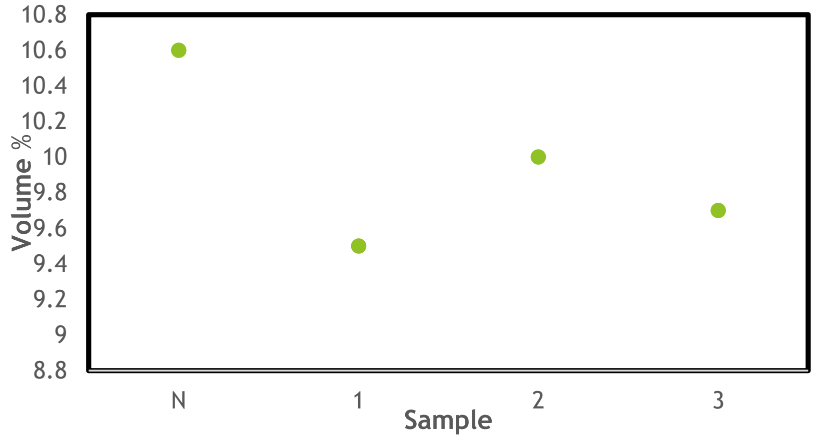


Fig 1 . Foam volume (%)

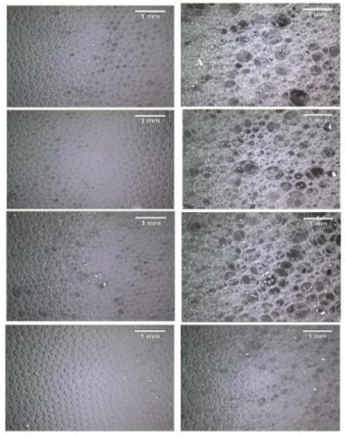


Fig 3 Digital images of foams after 1 min (left) and 20 min (right) of drainage.
A: atmosphere air, B: combination of argon + atmosphere, C: SF6, D: control

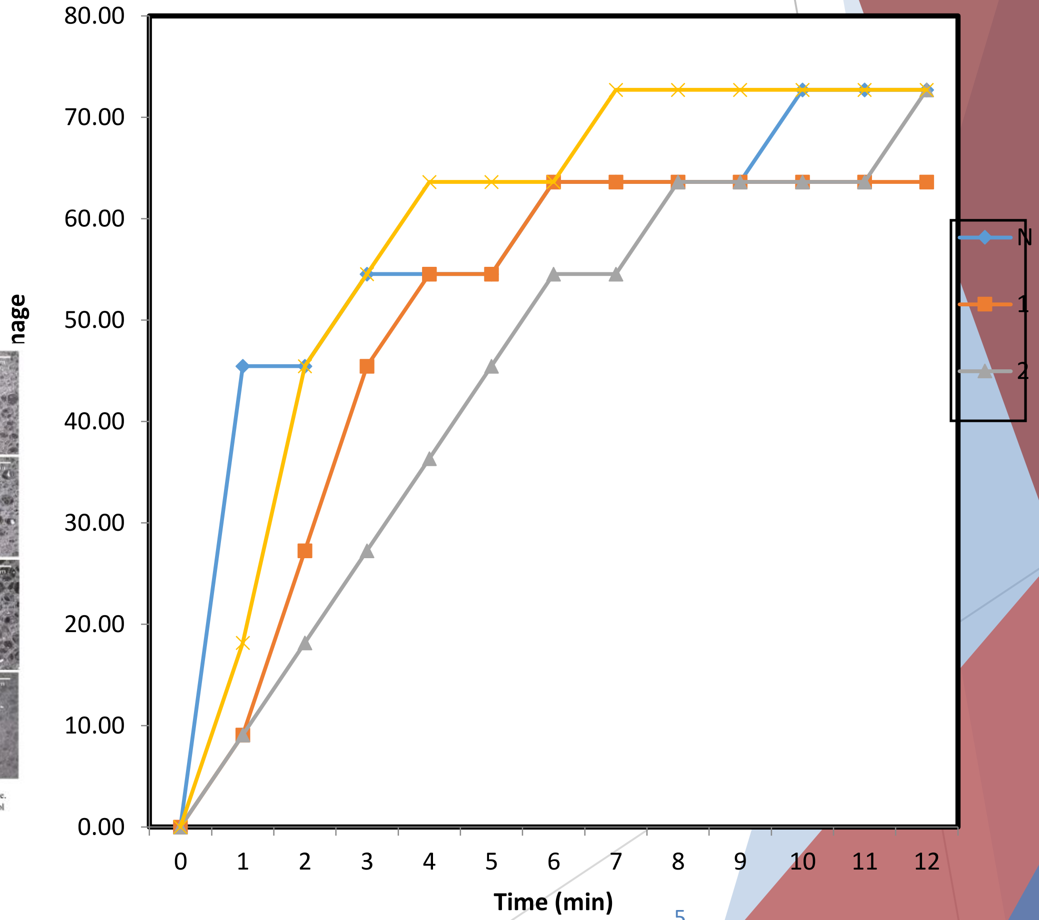


Fig 2. WPI Drainage foam

Results

Carbonyl group and free sulfhydryl group value results showed the increase in carbonyl group and decrease in free sulfhydryl group of protein by cold plasma treatment related to the oxidation process.

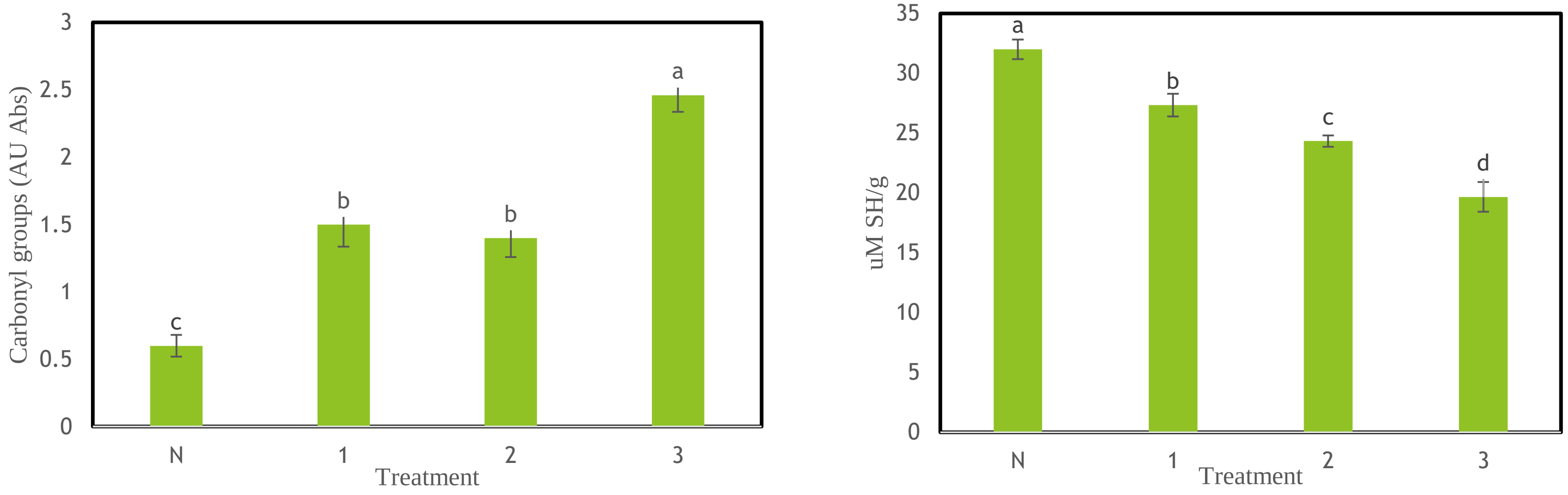


Fig 3. WPI Carbonyl group and Sulfhydryl group measurement

Results

To see the Hydrophobic changes of protein, spectrofluorometer was used. Results showed an increased in Intrinsic hydrophobicity and Fluorescence intensity in sample 2 (combination gas) that may be related to the changes in aromatic amino acids in protein by plasma. In contrast, the hydrophobicity in samples 1 and 3 was lower than in non-treated protein.

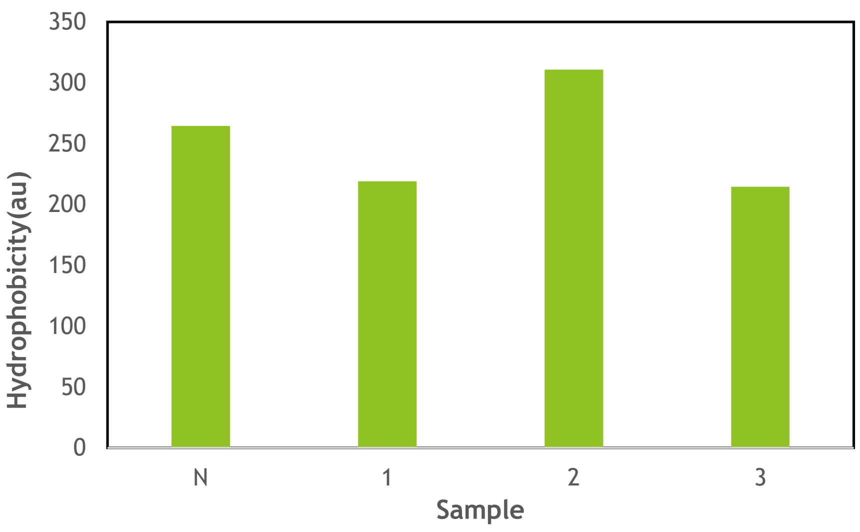


Fig 4. Intrinsic Hydrophobicity

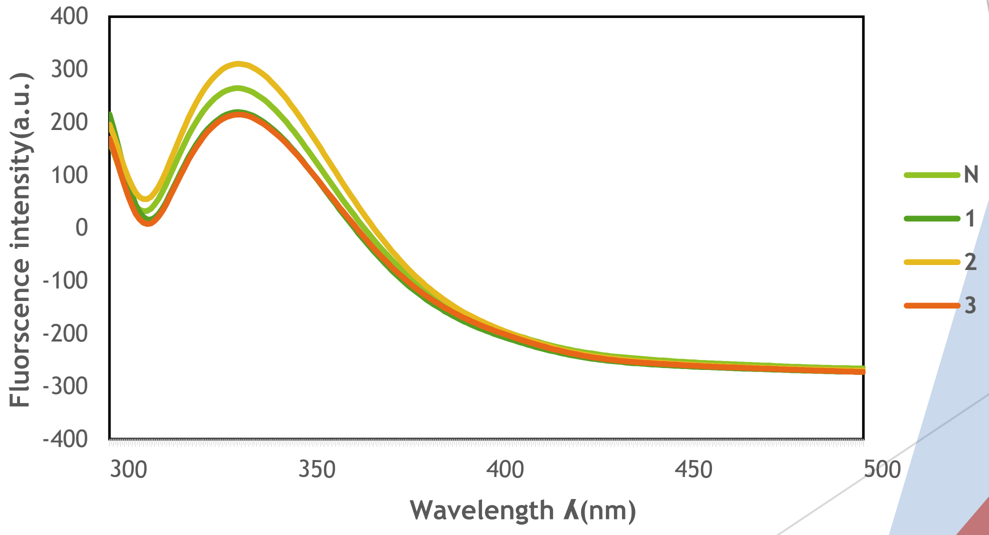
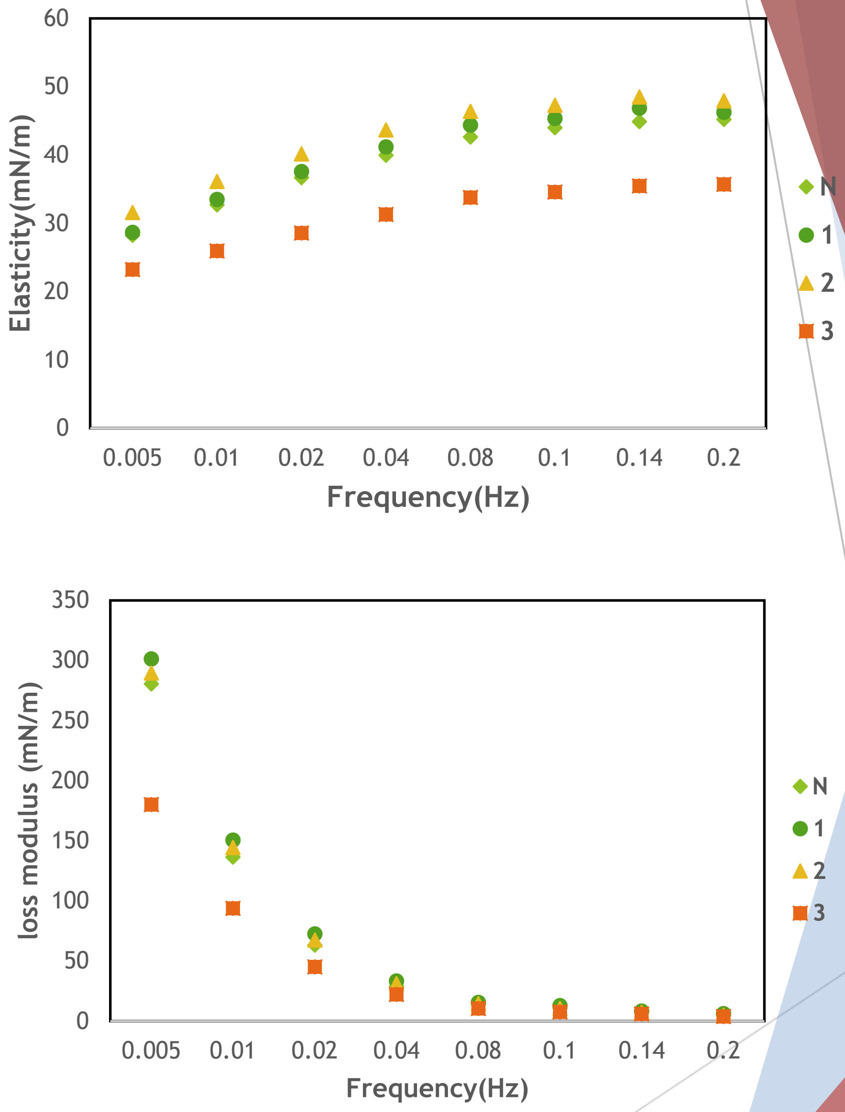


Fig 5. Fluorescence Intensity

Results

pressure isotherm showed an increase of pressure with increase of concentration of WPI.
Plotting Elasticity against frequency(0.01% wt. Concentration) indicated that cold plasma increased Elasticity of protein that could be related of structure changes of protein and aggregation.



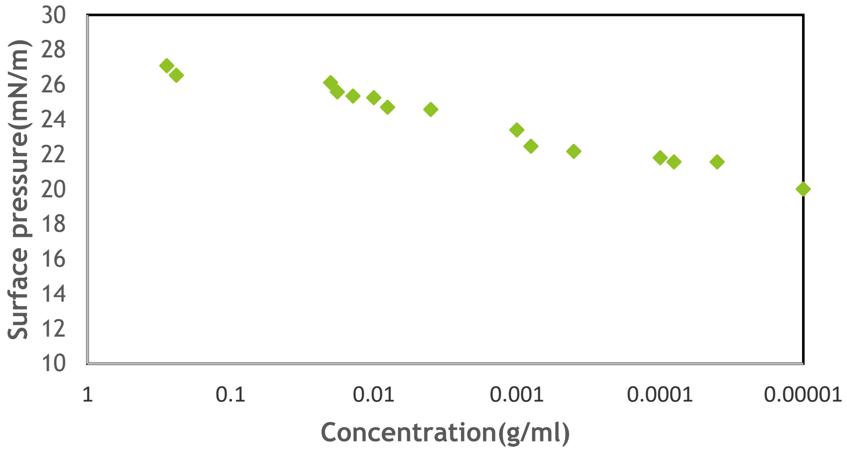


Fig 7. Surface Isotherm of WPI

Fig 6. Dilatational Elasticity and loss dilatational modulus of WPI

Results

Dynamic surface dilatational elasticity and surface pressure (measured by pendant drop) and thickness(measured by ellipsometer) for WPI samples (at 0.1%wt concentration) showed an increase in the Elasticity and thickness of surface protein film in the sample treated by combination gas cold plasma (2) and indicates more coverage of protein in the surface.

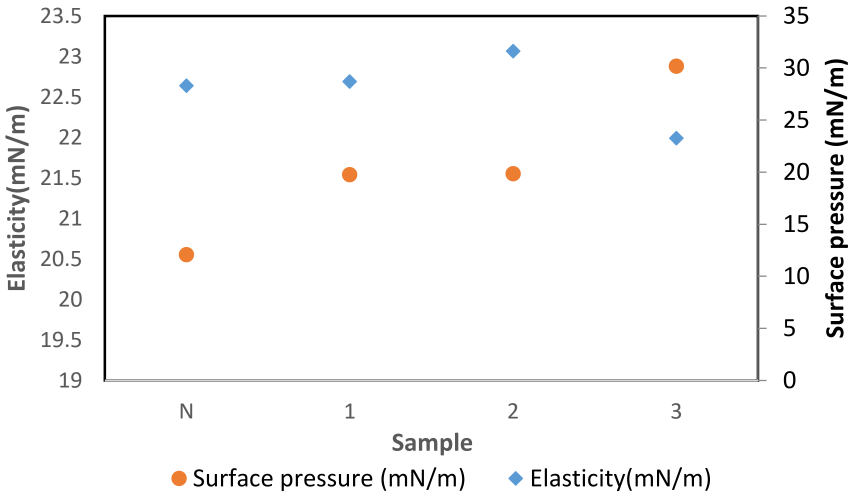


Fig 8. Dilatational Elasticity and Surface pressure of WPI(0.01%.wt)

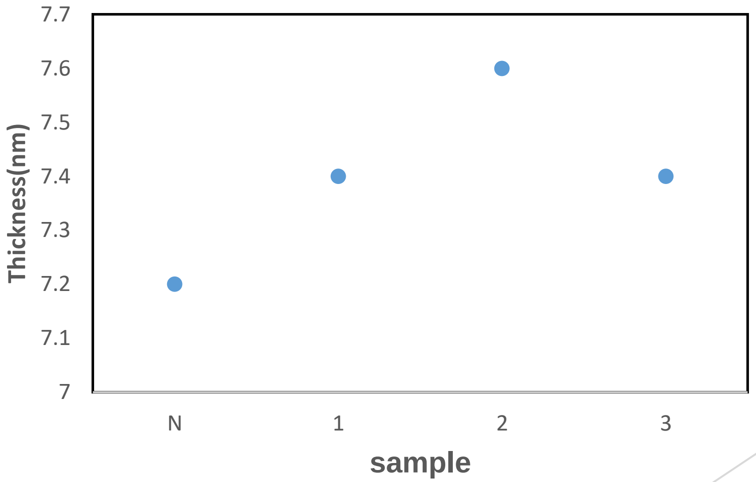


Fig 9. Surface thickness of WPI(0.1%.wt)

Conclusion

- In this study, interfacial adsorption and surface characteristics of WPI treated with cold plasma applying different gases were obtained at temperature 20 ℃ and pH 7.2 and ion strength of 0.05M.
- The results showed significant effects of cold plasma on the surface properties (as increasing in the surface elasticity, surface film thickness, foam stability, and surface pressure) of WPI.
- Among all gases used in this study, a combination of atmosphere and argon gas(50:50) was the most effective treatment on increasing surface interfacial properties and strength of WPI.