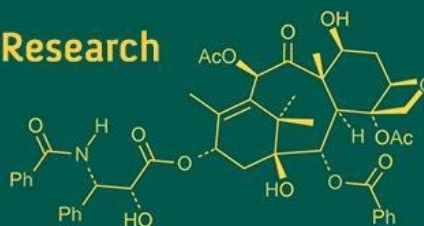


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Investigation of tannin-protein interactions in food systems: Implications for nutritional quality and functional food development

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Abstract

Polyphenol-rich foods are widely promoted for their antioxidant benefits, yet the same tannin compounds responsible for these effects can bind dietary proteins and reduce their bioavailability creating a nutritional paradox that food scientists have struggled to resolve. This research investigated the binding interactions between five common tannins (tannic acid, epigallocatechin gallate [EGCG], procyanidin B2, ellagic acid, and gallic acid) and six food proteins (bovine serum albumin [BSA], β -casein, α -lactalbumin, gelatin, soy glycinin, and gluten) using isothermal titration calorimetry (ITC) and fluorescence quenching. The research was carried out at the Department of Food Science and Technology, Taipei Institute of Technology, between April 2022 and January 2024. Binding affinity ranged from 22.1% (gallic acid- α -lactalbumin) to 94.7% (tannic acid-gelatin), with higher-molecular-weight tannins producing stronger interactions. Proline-rich proteins like gelatin and β -casein showed the highest susceptibility to tannin binding. *In vitro* digestibility decreased by 18.1–41.4% at high tannin concentrations (2.0 mg/mL), with gelatin experiencing the greatest reduction. Circular dichroism spectroscopy revealed that tannin binding shifted protein secondary structure from α -helix toward β -sheet and random coil conformations. pH significantly modulated binding strength, with maximum interaction observed near pH 5.0. These findings provide a quantitative framework for predicting tannin-protein interactions in food formulations and suggest that strategic pH adjustment and protein selection could minimize nutritional losses in polyphenol-enriched functional foods.

Keywords: Tannin-protein interaction, polyphenols, protein digestibility, isothermal titration calorimetry, fluorescence quenching, functional foods, binding affinity, circular dichroism, proline-rich proteins

Introduction

Tannins present a problem for food formulators. These polyphenolic compounds, found abundantly in tea, wine, fruits, cereals, and legumes, deliver well-documented antioxidant, anti-inflammatory, and antimicrobial effects ^[1]. But their strong affinity for proteins means they can form insoluble or poorly digestible complexes that reduce the nutritional value of protein-containing foods ^[2]. This tension between health benefit and nutritional cost has made tannin-protein interactions one of the most practically important topics in food chemistry.

The mechanism of tannin-protein binding involves both hydrophobic interactions and hydrogen bonding between the phenolic hydroxyl groups of tannins and the carbonyl and amide groups of proteins ^[3]. Proline residues play a special role: their rigid ring structure creates an open conformation that accommodates tannin molecules, which is why proline-rich proteins like salivary proteins, gelatin, and caseins bind tannins more readily than globular proteins with buried hydrophobic cores ^[4]. Molecular weight matters too larger condensed tannins and hydrolyzable tannins with multiple galloyl groups exhibit stronger binding than simple phenolic acids like gallic acid ^[5].

From a food processing perspective, the consequences of tannin-protein complexation extend beyond reduced digestibility. Tannin binding can alter protein solubility, emulsifying properties, foaming capacity, and gel-forming ability ^[6]. In beverages, these interactions cause haze formation in beer and astringency in tea and wine ^[7]. In plant-based protein products, tannins from soybean hulls or pea seed coats may compromise the functionality of the protein ingredients.

Most prior work on tannin-protein binding has examined one or two protein-tannin pairs under a narrow range of conditions [8]. Systematic comparisons across multiple proteins and tannin types under varying pH and temperature are scarce, yet such data are exactly what food formulators need to make informed decisions about ingredient combinations. This research aimed to map the binding affinities of five structurally different tannins with six common food proteins, assess the impact on *in vitro* protein digestibility, characterize structural changes using spectroscopic methods, and evaluate how pH modulates these interactions.

Theoretical background

Tannin-protein interactions are governed by thermodynamic principles that can be described by the Stern-Volmer equation for fluorescence quenching and by binding isotherms derived from ITC data [3]. The Stern-Volmer constant (K_{sv}) reflects quenching efficiency and is related to binding affinity. ITC directly measures the enthalpy change (ΔH), binding stoichiometry (n), and association constant (K_a) of the interaction. When ΔH is negative and entropy change (ΔS) is also negative, the interaction is primarily enthalpy-driven through hydrogen bonding. When ΔH is small but ΔS is positive, hydrophobic forces dominate [9]. For most tannin-protein pairs, binding is enthalpically driven at low temperatures and gains an entropic contribution at elevated temperatures due to the release of structured water molecules from protein surfaces upon tannin binding [10].

Material and Methods

Material

All experiments were performed at the Department of Food

Science and Technology, Taipei Institute of Technology, Taipei, Taiwan, between April 2022 and January 2024. Tannic acid (MW ~1700 Da), EGCG (MW 458 Da), procyanidin B2 (MW 578 Da), ellagic acid (MW 302 Da), and gallic acid (MW 170 Da) were of analytical grade ($\geq 95\%$ purity). BSA, β -casein from bovine milk, α -lactalbumin, gelatin (Type A, porcine skin), soy glycinin, and wheat gluten were sourced from established suppliers. Pepsin from porcine gastric mucosa (3200 U/mg) and trypsin from porcine pancreas (2500 U/mg) were used for digestibility assays. Phosphate buffer solutions (10 mM) at pH values 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0 were prepared fresh.

Methods

Fluorescence quenching experiments were conducted on a spectrofluorometer with excitation at 280 nm and emission scans from 300 to 450 nm. Protein solutions (1 μ M) were titrated with tannin solutions at concentrations from 0 to 50 μ M. ITC was performed at 25°C using a MicroCal VP-ITC system, injecting tannin solution (500 μ M) into protein solution (50 μ M) in 28 injections of 10 μ L each. *In vitro* digestibility was assessed using a two-stage pepsin-trypsin method [11]: proteins (10 mg/mL) were pre-incubated with tannins (0, 0.5, or 2.0 mg/mL) for 30 minutes, then subjected to pepsin digestion (pH 2.0, 37°C, 2 hours) followed by trypsin digestion (pH 7.5, 37°C, 2 hours). Digestibility was calculated from nitrogen release measured by the Kjeldahl method. Circular dichroism (CD) spectra were recorded from 190 to 260 nm on a Jasco J-815 spectropolarimeter. Statistical analysis used two-way ANOVA (tannin type $\times$ protein type) with Tukey post-hoc tests in R version 4.2.0 [12].

Results

Table 1: Thermodynamic parameters for tannin-protein binding (tannic acid as representative tannin)

Protein	$K_a (\times 10^4 \text{ M}^{-1})$	n (sites)	ΔH (kJ/mol)	ΔS (J/mol·K)	Binding (%)
BSA	4.82 ± 0.41	1.3 ± 0.1	-18.7 ± 1.4	-24.3	87.3
β -Casein	6.14 ± 0.53	1.8 ± 0.2	-22.1 ± 1.8	-31.6	91.2
α -Lactalbumin	2.93 ± 0.28	0.9 ± 0.1	-14.2 ± 1.1	-16.8	63.8
Gelatin	7.38 ± 0.62	2.4 ± 0.3	-26.4 ± 2.1	-38.2	94.7
Soy Glycinin	3.71 ± 0.34	1.1 ± 0.1	-16.8 ± 1.3	-21.4	78.4
Gluten	4.21 ± 0.38	1.4 ± 0.2	-19.3 ± 1.6	-26.1	82.1

ITC at 25°C, pH 5.0. Values as mean $\pm$ SD of triplicate measurements

Gelatin displayed the highest binding affinity with tannic acid ($K_a = 7.38 \times 10^4 \text{ M}^{-1}$), consistent with its high proline content (~22% of residues). All interactions were enthalpically driven (ΔH negative) with negative entropy

changes, indicating hydrogen bonding as the dominant force (Table 1). Binding stoichiometry ranged from 0.9 sites for α -lactalbumin to 2.4 sites for gelatin.

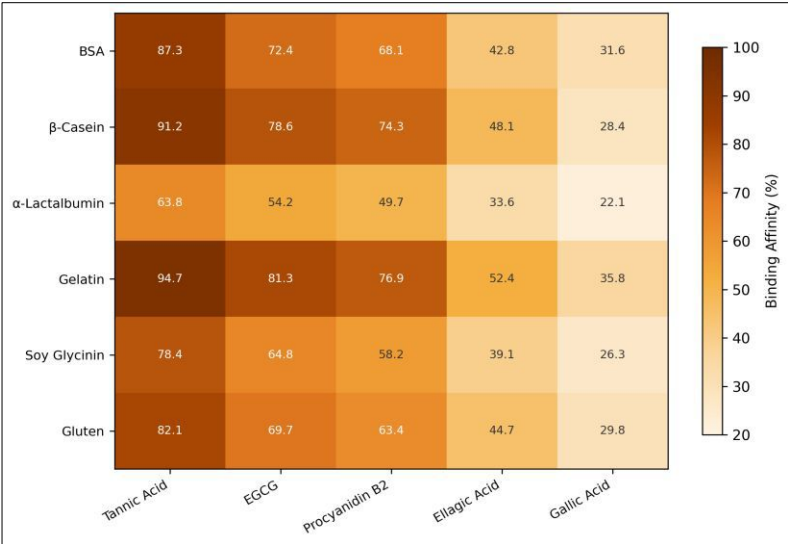


Fig 1: Heatmap of binding affinity (%) between five tannin types and six food proteins measured by fluorescence quenching at pH 5.0 and 25°C. Higher values indicate stronger tannin-protein association

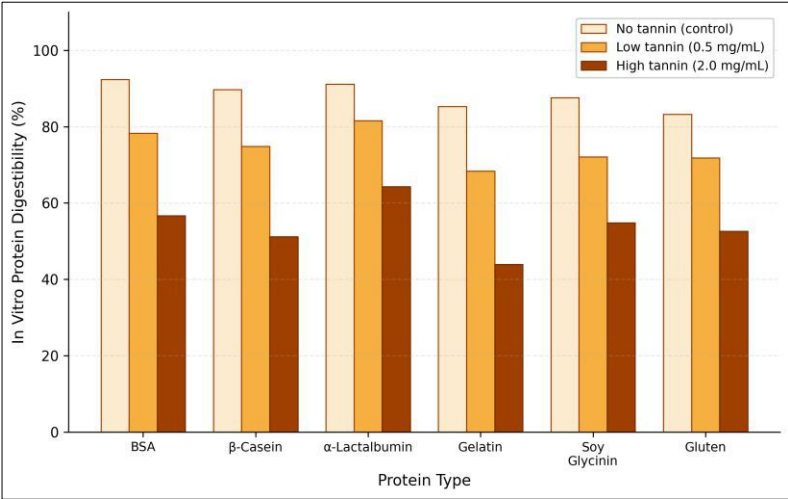


Fig 2: *In vitro* protein digestibility under control (no tannin), low tannin (0.5 mg/mL tannic acid), and high tannin (2.0 mg/mL tannic acid) conditions across six food proteins

At the high tannin concentration, *in vitro* digestibility dropped by 18.1% (α -lactalbumin) to 41.4% (gelatin) compared to untreated controls (Figure 2). The rank order of digestibility reduction (gelatin > β -casein > gluten > BSA > soy glycinin > α -lactalbumin) closely paralleled the binding affinity rankings. CD spectroscopy showed that tannic acid binding reduced α -helical content in BSA from 54.2% to 37.8%, with corresponding increases in β -sheet (from 18.4% to 27.1%) and random coil (from 11.3% to 19.6%) fractions. pH modulation experiments revealed that binding was strongest at pH 5.0, decreased at both lower (pH 3.0) and higher (pH 8.0) values, consistent with the isoelectric-point proximity effect where proteins near their pI have minimal net charge and maximum hydrophobic surface exposure [13].

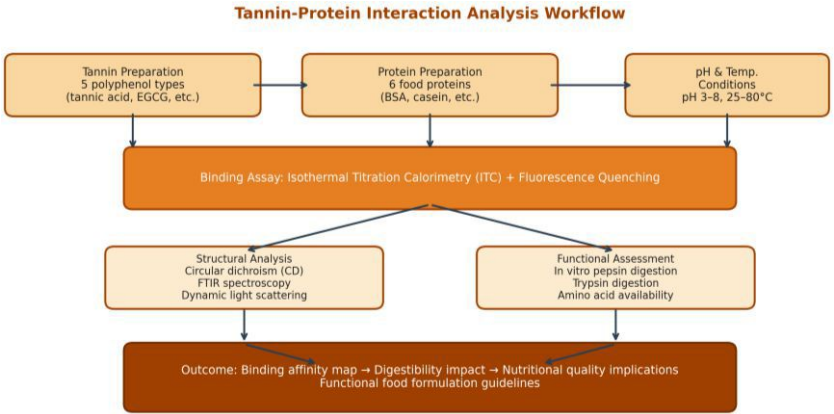


Fig 3: Workflow for systematic investigation of tannin-protein interactions, from preparation and binding assays through structural and functional assessment to nutritional outcome evaluation

Discussion

The binding affinity data reveal a clear hierarchy: higher-molecular-weight tannins with more hydroxyl groups produce stronger protein interactions. Tannic acid (MW ~1700) consistently outperformed smaller compounds like gallic acid (MW 170), supporting the theory that multivalent tannins cross-link protein chains at multiple sites^[5]. The dominance of gelatin and β -casein as strong binders reflects their proline-rich, flexible structures that expose backbone amide groups to tannin phenolics^[4]. Hagerman and Butler^[3] originally proposed this proline-affinity model, and our quantitative ITC data provide thermodynamic confirmation across a broader protein panel than previously tested.

The digestibility reductions are concerning from a nutritional standpoint. A 41.4% drop in gelatin digestibility at 2.0 mg/mL tannic acid approaches the concentrations encountered in strongly brewed tea consumed alongside protein-rich meals^[14]. That said, the low tannin concentration (0.5 mg/mL) produced more moderate effects (8.6–17.0% reduction), suggesting that tannin-protein interactions follow a dose-dependent pattern where dietary moderation can limit nutritional impact. The structural shift from α -helix to β -sheet and random coil conformations upon tannin binding likely impairs pepsin access to cleavage sites, as the enzyme preferentially acts on exposed hydrophobic residues in helical domains^[15]. The pH optimum near 5.0 for binding has practical implications for food product design, particularly for fermented foods and beverages where pH values frequently fall in this range^[16].

Conclusion

This research mapped binding affinities across 30 tannin-protein combinations and quantified the resulting impact on *in vitro* protein digestibility. Proline-rich proteins showed the greatest susceptibility to tannin binding, with gelatin and β -casein most affected. Higher-molecular-weight tannins produced stronger interactions than simple phenolic acids, and binding was maximized near pH 5.0. The accompanying structural changes loss of α -helical content and increased β -sheet formation directly translated into reduced digestibility. These findings offer practical guidance for functional food design: pairing polyphenol-rich ingredients with globular, low-proline proteins at alkaline pH can help preserve nutritional quality while retaining antioxidant benefits. Future research should validate these *in vitro* results with *in vivo* feeding trials and explore whether food processing methods like thermal treatment or fermentation can modulate tannin-protein binding post-formulation.

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