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Food Science and Technology Department

B.Sc. in Food Science and Technology

Special Graduation Project

Functional Properties of Sunflower Flour as a Function of Food-grade Thiols and pH

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Table of Contents

List of Tables

List of Figures

Abstract

Sunflower (*Helianthus annuus*) flour is a promising non-GMO and allergen-free protein source with increasing relevance as an alternative to conventional oilseed soy proteins. However, a significant challenge that limits its industrial application is the undesirable greening that occurs during alkaline extraction. This greening is primarily caused by the oxidation of chlorogenic acid (CGA), which reacts with protein amino groups, to form green trihydroxybenzacridine (TBA) pigments. This study evaluated the effect of two food-grade thiol compounds: Cysteine (Cys) and Glutathione (GSH), at concentrations of 0.0, 2.8, and 5.6 mM and two pH levels (9 and 11), on the color, functional properties, protein content, chlorogenic acid content, and Maillard reaction products of sunflower protein powders. A Factorial Complete Randomized Design (CRD) was used to evaluate the effect of thiol type, thiol concentration and pH on greening (-a* values), browning index (BI), functional properties, CGA content, protein content, and Maillard reaction products, including advanced glycation end-products (AGEs), hydroxymethylfurfural (HMF), and Fluorescence of Advanced Maillard Products and Soluble Tryptophan (FAST) Index. Compositional analysis for protein content (73.10-84.8%) and CGA remained unchanged. Results showed that 5.6 mM cysteine at pH 11 effectively lowered greening and enhanced browning. Functional properties such as water and oil holding capacities, nitrogen solubility, and surface hydrophobicity were not significantly affected, as well as Maillard reaction products. These findings support the use of thiol-assisted extraction to improve the visual quality of sunflower protein without compromising functionality.

Keywords: Alkaline extraction, cysteine, glutathione, greening, sunflower protein, thiol compounds.

Resumen

La harina de girasol (Helianthus annuus) es una fuente de proteína prometedora, libre de OGM y alérgenos, con creciente relevancia como alternativa a las proteínas convencionales de soya. Sin embargo, un desafío importante que limita su aplicación industrial es el enverdecimiento indeseable que se produce durante la extracción alcalina. Este enverdecimiento se debe principalmente a la oxidación del ácido clorogénico (ACG), que reacciona con los grupos amino de las proteínas para formar pigmentos verdes de trihidroxibenzacridina (TBA). Este estudio evaluó el efecto de dos compuestos de tiol de grado alimenticio: cisteína (Cys) y glutatión (GSH), en concentraciones de 0.0, 2.8 y 5.6 mM y dos niveles de pH (9 y 11), sobre el color, las propiedades funcionales, el contenido de proteína, el contenido de ácido clorogénico y los productos de la reacción de Maillard de las proteínas de girasol en polvo. Se utilizó un Diseño Completamente al Azar (DCA) con un arreglo factorial de tratamiento para evaluar el efecto del tipo de tiol, su concentración y el pH sobre el enverdecimiento (valores -a*), el índice de pardeamiento (IP), las propiedades funcionales, el contenido de ACG, el contenido de proteína y los productos de la reacción de Maillard, incluyendo los productos finales de glicación avanzada (AGE), el hidroximetilfurfural (HMF) y el índice de fluorescencia de productos avanzados de Maillard y triptófano soluble (FAST). El análisis composicional del contenido proteico (73.10-84.8%) y el CGA no presentaron cambios. Los resultados mostraron que 5.6 mM de cisteína a pH 11 redujeron eficazmente el enverdecimiento y potenciaron el pardeamiento. Las propiedades funcionales, como la capacidad de retención de agua y aceite, la solubilidad en nitrógeno y la hidrofobicidad superficial, no fueron afectadas significativamente, al igual que los productos de la reacción de Maillard. Estos resultados respaldan el uso de la extracción asistida por tiol para mejorar la calidad visual de la proteína de girasol sin comprometer su funcionalidad.

Palabras clave: Cisteína, compuestos tiolados, glutatión, proteína vegetal, reacción de Maillard.

Introduction

Sunflower (*Helianthus annuus*) flour is an alternative protein source to the traditional oilseed soy proteins with increasing relevance in the food industry due to its high protein content, non-GMO status, and absence of major allergens (Wildermuth et al., 2016). However, a significant limitation in its commercial application is the potential undesirable greening that occurs during alkaline extraction. Chlorogenic acid is a key phenolic compound found in high concentrations of 2–5% in sunflower protein s that contributes to unwanted pigmentation during alkaline processing (González-Pérez, 2003). This discoloration is attributed to the oxidation of chlorogenic acid (CGA) and its subsequent reaction with protein amino groups, forming green trihydroxy benzacridine (TBA) derivatives (Liang y Were, 2020). To mitigate greening, thiol-containing compounds such as L-cysteine (Cys) and reduced glutathione (GSH) have been investigated as alternatives to conventional de-greening methods in industry such as oxygen exclusion, acidic precipitation, and organic solvent extraction used during sunflower protein isolation (Ishii et al., 2021; Pickardt et al., 2011). The thiol compounds are effective de-greening ingredients due to their ability to interact with oxidized CGA and prevent pigment formation, preventing its conjugation with proteins and thereby inhibiting the formation of green pigments (Liang & Were, 2020; Pepra-Ameyaw et al., 2023). A critical challenge in the field is to ensure that strategies that prevent greening do not negatively impact protein functionality.

Given that the extent to which thiol addition impacts functional properties of sunflower protein remains unclear, particularly under varying pH extraction conditions. This research goal was to determine how different concentrations of thiols (Cys and GSH) at pH 9 and pH 11 influence protein structure, solubility, water-holding capacity (WHC), oil-holding capacity (OHC), color stability, and advanced glycation end-products (AGEs) formation. Higher pH can enhance protein extraction efficiency but may also induce structural modifications that influence protein functionality (Kaur &

Ghoshal, 2022). This study addressed this knowledge gap by evaluating the influence of thiol concentration and pH on the functional properties of sunflower protein isolates.

The null hypothesis declared was that increasing thiol concentration would influence protein functionality and pigmentation in sunflower protein isolates. Thiols could modulate protein structure by altering disulfide bond interactions, potentially improving protein solubility and water-holding capacity (Karefyllakis et al., 2018). Cysteine's role in influencing protein structure and functionality has been demonstrated in food systems where its thiol group promotes disulfide bond exchange, contributing to enhanced protein folding and intermolecular interactions. Specifically, cysteine was shown to increase hydrophobicity and potentially improve structural stability and water-holding capacity when incorporated into sunflower protein extraction systems (Kinsella, 1979). Increasing GSH concentration was expected to result in higher protein solubility, likely through its reduction of disulfide bonds that otherwise contribute to protein aggregation (Salgado et al., 2012).

in this study, thiol addition was also evaluated for its role in mitigating pigment formation and preserving protein functionality. It was also hypothesized that thiol–CGA quinone conjugates formation of green pigment formation will be lowered by GSH and cysteine, reacting with chlorogenic acid quinones, forming colorless thiol–quinone conjugates (Ishii et al., 2021). It was also hypothesized that increasing thiol concentration can enhance the formation of fluorescent Advanced Glycation End-Products (AGEs) by facilitating nucleophilic addition to glucose intermediates (Lund y Ray, 2017).

Materials and Methods

Location

The entire study was conducted at Chapman University, California, United States of America.

Materials

Sunflower flour (Suntein™, 32% protein) was obtained from Red River Commodities (Fargo, ND, USA). L-cysteine hydrochloride and glutathione thiol compounds (98% purity) were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade acetonitrile and water, used for chromatographic separations, were obtained from Thermo Fisher Scientific (Hampton, NH, USA). The Radleys Carousel 12 Plus Reaction Station™ (Essex, UK) was employed for the controlled thermal generation of Advanced Glycation End-products (AGEs). Carrez reagents I and II used to remove interfering macromolecules during sample clarification was obtained from Thermo Fisher Scientific (Hampton, NH, USA). A Phenomenex Luna 3 µm C18(2) 100 Å, 150 × 4.5 mm column (Torrance, CA, USA) was used for HPLC analysis of 5-hydroxymethylfurfural (HMF) and chlorogenic acid (CGA). All other reagents were analytical or HPLC grade.

Experimental Design

A factorial Complete Randomized Design was employed to evaluate the effect of thiol type (Cysteine vs. Glutathione), thiol concentration (2.8, 5.6 mM), control (pH 9 + 0.0mM thiols and pH 11 + 0.0mM thiols) and pH (pH 9 vs. pH 11) on the functional properties of sunflower flour resulting in 10 different treatment combinations, each defined by the interaction of these three factors:

Thiol Type (2 levels): Cysteine (Cys) and Glutathione (GSH).

Thiol Concentration (2 levels): 2.8 mM, and 5.6 mM.

pH Condition (2 levels): pH 9 and pH 11.

Controls (2): pH 9 + 0.0mM thiols and pH 11 + 0.0mM thiols

Treatments: (2 thiol types × 2 Concentrations × 2 pH conditions) + 2 controls = 10 treatments. Each treatment was replicated three times, resulting in a total of 30 experimental units

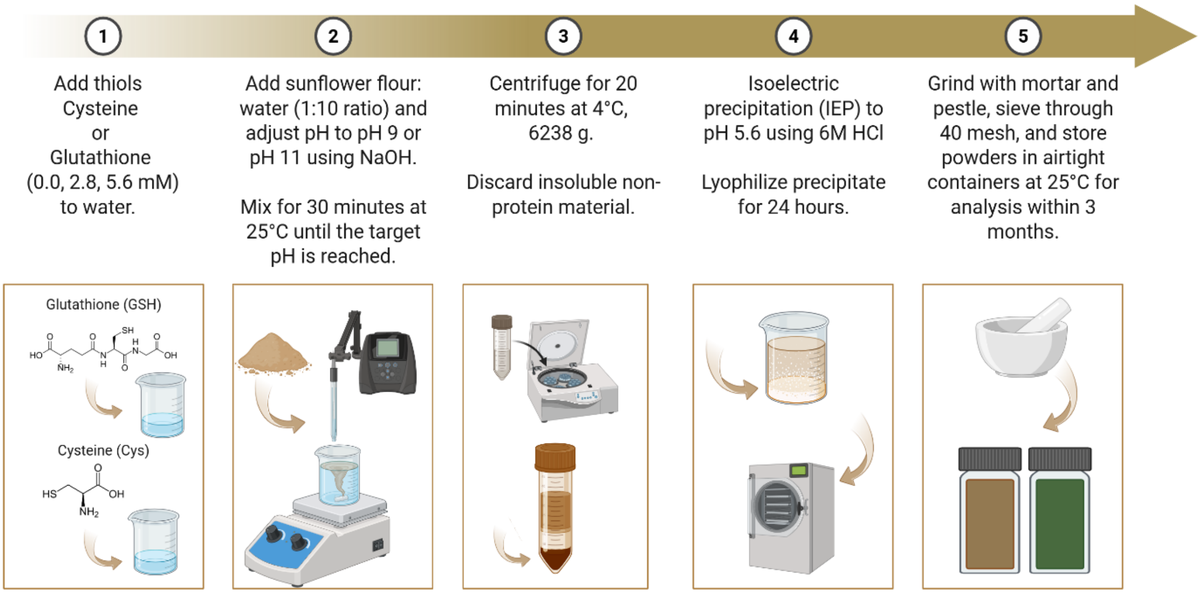
Sunflower Isolate Preparation

Alkaline-Isoelectric point (IEP extraction process of Ishii et al., 2021 was used to prepare sunflower isolate from Suntein™ sunflower flour (Red River Commodities, Fargo, ND, USA), which contained 32% protein. Thiol solutions were prepared by dissolving 0.0-, 2.8-, and 5.6-mM L-cysteine hydrochloride or reduced glutathione (GSH) in 450 mL of deionized water in 2-liter glass beakers. The solutions were stirred continuously at 850 rpm using Cimarec+™ Stirring hotplates (Thermo Fisher Scientific, Hampton, NH, USA). Subsequently, 50 g of Suntein™ sunflower flour was added to each thiol solution under constant stirring (Figure 1). The protein to water ratio of 1:10 was selected in accordance to Ishii et al., (2021), (Pickardt et al., (2011) and (Albe Slabi et al., 2020) ratios used. The mixtures were maintained at 25°C and adjusted to pH 9 or 11 by gradual addition of 6 M NaOH over 30 minutes, with pH monitored every 10 minutes. After an initial stabilization period (~10 minutes), stirring continued for another 30 minutes with periodic pH adjustments to maintain the target pH.

The mixtures were then centrifuged at 6238 g and 4°C for 20 minutes using a Thermo Scientific X1R centrifuge (Osterode am Harz, Germany) to separate solubilized proteins. The supernatants underwent isoelectric precipitation by adjusting the pH to 5.6 with 6 M HCl, followed by centrifugation under the same conditions. The precipitated protein isolates were lyophilized under vacuum for approximately 24 hours, with a freezing step at −30°C for 9 hours and subsequent gradual sublimation at 20°C, 40°C, 60°C, 90°C, and 120°C over 15 hours using a Harvest Right freeze dryer (Utah, USA). The dried sunflower protein isolates were weighed, ground with a mortar and pestle, and passed through a #40 mesh sieve (0.841 mm) prior to further analysis.

Figure 1

Sunflower flour protein powders alkaline extraction.



1. Add thiols Cysteine or Glutathione (0.0, 2.8, 5.6 mM) to water.
2. Add sunflower flour: water (1:10 ratio) and adjust pH to pH 9 or pH 11 using NaOH. Mix for 30 minutes at 25°C until the target pH is reached.
3. Centrifuge for 20 minutes at 4°C, 6238 g. Discard insoluble non-protein material.
4. Isoelectric precipitation (IEP) to pH 5.6 using 6M HCl. Lyophilize precipitate for 24 hours.
5. Grind with mortar and pestle, sieve through 40 mesh, and store powders in airtight containers at 25°C for analysis within 3 months.

Greening and Browning Color Effect of pH, Thiol Type and Concentration

The lightness (L* from 0 for darkness to 100 for lightness), redness (+a*) and greenness (-a*), and yellowness (+b*) and blueness (-b*) values of sunflower flour were measured using a Hunter L*, a*, b* spectrophotometer (CM-2500D, Konica Minolta, Inc., Tokyo, Japan). The browning index (BI) was calculated from the Hunter L*a*b** values using Equation [1] provided by Maskan (2001).

$$BI = 100 \times \left[\left(\frac{X-0.31}{0.17}\right)\right] \qquad [1]$$

where:

$$X = (a* + 1.75L) \,/\, (5.645L + a* - 3.012b*)$$

Protein and Chlorogenic acid Quantification

Protein Quantification by Kjeldahl Analysis

Kjeldahl Analysis was performed on a Kjeltec™ 8100 unit with 0.20 g of sunflower protein powder weighed into a digestion tube. Then, 7 mL of concentrated sulfuric acid and one catalyst tablet were added. The tube was placed on a digestion block set to 375°C for 50 minutes and allowed to digest until the solution became clear and neon green. After digestion, the tubes were removed and cooled. The distillation system was set up, and a boric acid solution was placed in the receiving flask. The Kjeldahl tube was positioned, and steam distillation was carried out. For titration, 0.05 M HCl was used as the titrant, and the volume of HCl used was recorded. A N factor of 5.75 following the protocol outlined by (Nielsen, 2010) was used.

Quantification of Chlorogenic Acid (CGA) by HPLC

Sunflower protein powder (0.20 g) was weighed into a beaker. Then, 10 mL of 70% ethyl alcohol (ethanol) was added, followed by the addition of stir bars. The mixture was stirred for approximately 1 hour (Cimarec+™ Stirring hotplates, Thermo Fisher Scientific, Hampton, NH, USA).

After extraction, the mixtures were transferred to Eppendorf tubes and centrifuged (Thermo Scientific X1R centrifuge). The supernatant was then filtered using 0.45 µm syringe filters.

Next, 100 µL of the filtered supernatant was measured into HPLC vials, and 900 µL of HPLC-grade water was added. The vials were sealed and then transferred to the 1260 Agilent HPLC system.

Chlorogenic acid quantification was carried out using an external standard curve created from 0 to 0.1 mM CGA. The CGA solutions were prepared by serial diluting a 0.1 mM CGA stock solution (0.0354 g CGA in 10 mL HPLC-grade water). CGA was quantified using an Agilent 1260 series HPLC instrument (Santa Clara, CA, USA). The column used was a Phenomenex Luna 3 µm C18(2) 100 Å, 150 × 4.5 mm column and the column temperature was set to 30°C. The detection wavelength was set at 320 nm, and the flow rate was 0.3 mL/min. Mobile phase A was HPLC-grade water with 0.1% formic acid, and mobile phase B was HPLC-grade acetonitrile with 0.1% formic acid.

Water Holding Capacity (WHC), Nitrogen Solubility Index (NSI), and Oil Holding Capacity

The water and oil holding capacities of the protein fractions were determined as outlined by (Sosulski and McCurdy, 1987). Approximately 0.10 g of the protein powder was mixed with 10 mL of water or sunflower oil in a centrifuge tube. The mixture was vortexed for 10 seconds every 5 minutes over a 30-minute period to ensure thorough interaction. After mixing, the protein powders were centrifuged, and the supernatant was carefully decanted. The remaining pellet was dried using a HarvestRight Freeze Dryer and then weighed and formulas used for WHC, OHC and NSI where[2] [3] & [4]:

Water Holding Capacity (WHC)

$$WHC = (Mwet\ pellet - Mdry\ pellet)\ /\ Mdry\ pellet\ [g\ water/g\ dry\ pellet] \qquad [2]$$

Where Mwet pellet is the mass of the wet pellet, and Mdry pellet is the mass of the dry pellet. (Jia et al., 2022)

Oil Holding Capacity (OHC)

$$OHC(g/g) = msmfs - ms \qquad [3]$$

Where:

msfsm is the weight of the fatted pellet (g),

ms is the initial weight of the dry sample (g),

OHC is expressed as grams of oil retained per gram of dry matter (DM). (Jia et al., 2022)

Nitrogen Solubility Index (NSI)

$$Nitrogen\ solubility\ (\%) =$$

$$(Supernatant\ nitrogen\ content/Total\ sample\ nitrogen\ content) \times 100 \qquad [4]$$

Surface Hydrophobicity

Protein powders were dissolved in a 0.01M phosphate buffer at pH 7.4, vortexed, and centrifuged at 10,000 rpm for 10 minutes. 5 µL of -anilinonaphthalene-8-sulfonic acid (ANS) probe (8 mM stock solution) was added to achieve a final concentration of 20 µM. After incubating for 15-30 minutes at room temperature, fluorescence intensity was measured at an excitation wavelength of 390 nm and an emission wavelength of 470 nm. Surface hydrophobicity was calculated as the relative fluorescence intensity (RFI), subtracting the fluorescence of the buffer from that of the protein solutionfollowing the method by Y. Zhang y Xiao, (2025).

Quantification of 5-Hydroxymethylfurfural (HMF) by HPLC

Quantification of 5-hydroxymethylfurfural (HMF) was performed using an Agilent 1260 Series HPLC. A total of 0.20 g of sunflower powder was suspended in 10 mL of Milli-Q water, vortexed, and cooled at −20 °C for 15 minutes. The mixture was centrifuged at 22,000 × g for 15 minutes at 0 °C. The resulting supernatant was collected and stored at 4 °C until analysis.

For sample clarification, 1.5 mL of the water extract was mixed with 50 µL of Carrez Solution I (aqueous potassium hexacyanoferrate(II)) and 50 µL of Carrez Solution II (aqueous zinc sulfate), (Omitting either Carrez Solution I or II could result in incomplete clarification, leading to potential interference in the HPLC analysis and inaccurate quantification of HMF), vortexed, and centrifuged again at 22,000 × g for 15 minutes at room temperature. After filtering the supernatant through a 0.45 µm nylon syringe filter, 100 µL was transferred into an HPLC vial for chromatographic analysis using a Luna C18 column (150 mm length × 4.6 mm internal diameter × 5 µm particle size; Phenomenex, Torrance, CA, USA). The column temperature was maintained at 40°C. An isocratic elution was applied using 20% acetonitrile and 80% HPLC water at a flow rate of 0.50 mL/min. Detection of HMF was carried out at 285 nm and 277 nm.

Furfural is commonly detected at a wavelength of 280 nm in HPLC analysis; similar to HMF, furfural has a strong absorption in the UV range, particularly around 277-280 nm. The retention time (RT) for HMF under these conditions was 4.197 minutes. This protocol was adapted from (Pucci et al., 2024) with methodological modifications (the column used), based on the procedure described by (Favre et al., 2020) for HMF and furfural quantification.

Fluorescence of Advanced Maillard Products and Soluble Tryptophan (Fast) Index Measurement

The FAST index as outlined by (Zieliński et al., 2010), was used to assess changes resulting from combined effects of thiol type, pH and heat treatment on the Maillard reaction , and was calculated as per Equation 5:

$$FAST\ index = 100 \times (FIC/TrpFL) \tag{5}$$

Where:

FIC refers to the fluorescence of free intermediate carbonyls (Maillard reaction products), and

TrpFL refers to the fluorescence of soluble tryptophan.

To measure fluorescence for tryptophan (Trp), an excitation wavelength of 290 nm, and emission wavelength of 340 nm was used. For advanced Maillard products (FAMP), fluorescence was measured at an excitation of 330 nm and emission at 420 nm.

Total Fluorescent Advanced Glycation End Products (AGEs)

Sunflower powder (0.20 g) and 0.10 g of D-(+)-glucose anhydrous (≥99%, Thermo-Fisher Scientific, Hampton, NH, USA) were first suspended in 10 mL of deionized water before incubating in a Radleys Carousel 12 Plus Reaction Station™ (Essex, UK) at 50°C to promote Maillard reactions and AGE formation. Powder mixtures were taken out after 4 hours, 8 hours, and 24 hours of incubation to evaluate the progression of AGE formation over time. The fluorescence intensity for each time point was measured with an excitation wavelength of 370 nm (bandwidth: 20 nm), and emission was recorded at 440 nm (bandwidth: 20 nm) using a Spark® multimode microplate reader (Tecan Group Ltd., Männedorf, Switzerland).

Results and Discussion

Effect of pH and Thiol Type on yield, and Color Reactants (Chlorogenic Acid and Protein) Content

The protein yield ranged from 10.53 to 17.9 (Table 1). Protein yielding increased with higher extraction pH, as samples at pH 11 consistently showed greater values than those at pH 9. This aligned with (Ishii et al., 2021) who reported that alkaline pH enhances solubilization of helianthinin, leading to higher yields. Among thiols, moderate concentrations (≈2.8 mM) of both cysteine and glutathione gave the highest yielding, while higher levels (5.6 mM) reduced recovery, due to possible aggregation or structural changes.

Table 1

Alkaline pH, Isoelectric Point (IEP), and Yield, of Sunflower Flour Protein Powders.

pH Level	Thiol type and concentration	Alkaline pH	IEP	Protein yield (%)
pH 9	0.0 mM Thiols	9.12 ± 0.12	5.35 ± 0.34	11.34 ± 0.07
	2.8 mM GSH	9.23 ± 0.21	4.63 ± 0.66	12.44 ± 0.42
	5.6 mM GSH	9.03 ± 0.11	5.57 ± 0.07	10.53 ± 1.42
	2.8 mM Cys	9.14 ± 0.18	5.31 ± 0.31	10.85 ± 0.15
	5.6 mM Cys	9.21 ± 0.33	5.58 ± 0.05	11.45 ± 1.03
pH 11	0.0 mM Thiols	11.05 ± 0.10	5.66 ± 0.07	15.83 ± 0.81
	2.8 mM GSH	10.89 ± 0.09	5.67 ± 0.05	17.04 ± 0.20
	5.6 mM GSH	11.05 ± 0.14	5.41 ± 0.17	17.68 ± 0.47
	2.8 mM Cys	10.96 ± 0.08	5.56 ± 0.16	17.94 ± 0.47
	5.6 mM Cys	10.90 ± 0.13	5.53 ± 0.07	15.56 ± 0.25

Note. IEP: Isoelectric point; Cys: Cysteine; GSH: glutathione.

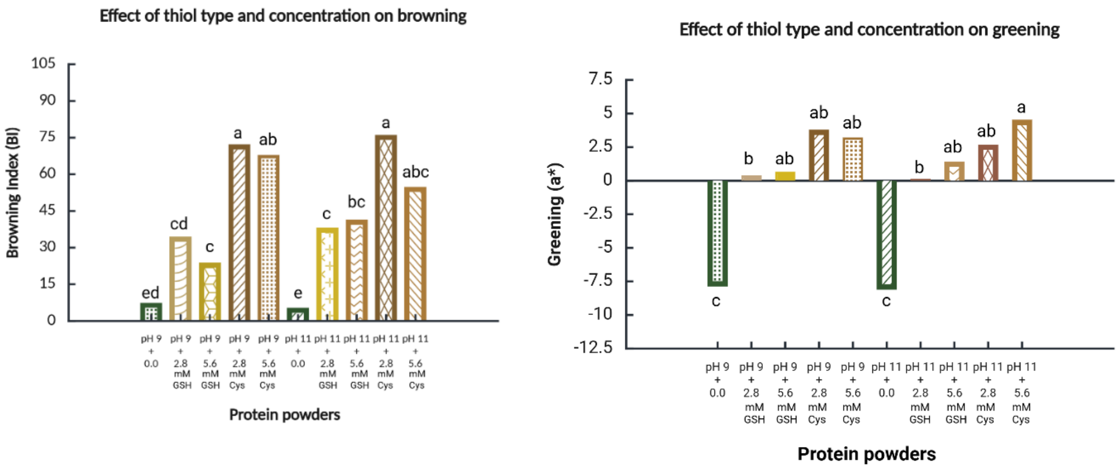
Effect of pH and Thiol Type on Browning Intensity and Greening

The General Linear Model (GLM) evaluating the effects of thiol type and thiol concentration at two different pH levels (pH 9 and pH 11) revealed a highly significant effect of thiol treatment and pH on greening, as expressed by a* values ($p < 0.0001$). With a strong model fit ($R^2 = 0.93$); the results confirm that both thiol treatment and pH significantly influenced the degree of green coloration. Tukey's HSD test identified clear differences among treatment groups, with the most intense greening (i.e., lowest a* values) observed in the absence of thiols at a value of −7.88 at pH 9 and −8.11 at pH 11 (Figure 2). These a*values were significantly lower (pH 9 and 11 at 0.0 mM thiols, Figure 2B) than all thiol-treated groups, indicating that thiol omission leads to unmitigated greening, likely due to increased quinone accumulation and pigment formation in alkaline environments (Ishii et al., 2021; W. Zhang et al., 2019). Conversely, 5.6 mM cysteine at pH 11 resulted in the highest a* value (4.55), statistically distinct (Figure 2B), indicative of the strongest greening mitigation among all tested conditions. Cysteine treatments at 2.8 or 5.6 mM at pH 9, as well as 2.8 mM at pH 11also demonstrated effective mitigation, with a* values ranging from 3.2 to 3.8. As illustrated in Figure 2, the higher a* values with cysteine, especially at 5.6 mM and pH 11, confirm Cysteine role in significantly lowering greening in sunflower protein extracts.

While adding Glutathione to powders mitigated greening compared to controls, exhibiting lower a* values (0.14 to 0.68), GSH had less effective greening mitigation compared to Cysteine. These findings reflect known differences in reactivity between the two thiols. Cysteine, due to its low molecular weight and high nucleophilicity, may reacts more rapidly with oxidized phenolics than glutathione, thereby limiting green pigment formation more effectively than GSH (Ishii et al., 2021)(Liang & Were, 2020).

Figure 2

Effect of thiol type & concentration on Browning Index (BI) and Greening (a) values*



(A) Browning Index (BI)

(B) Greening (a*)

The GLM analysis showed a significant effect (*p* < 0.0001, $R^2 = 0.89$) of treatments on the browning index, indicating that thiol type and pH combinations influenced browning.

The highest BI was observed with 2.8 mM cysteine at pH 11 (76.13) and at pH 9 as well (72.21), followed by 5.6 mM cysteine at pH 9 (67.94), showing no significance difference between them. In contrast, powders without thiols exhibited the lowest browning with a BI of 7.48 at pH 9 no thiols and 5.39 at pH 11 no thiols. These findings align with (Ishii et al., 2021) findings in which thiol addition, particularly Cysteine, enhanced Maillard-browning under alkaline conditions at the expense of greening. When thiols are present, they preferentially bind quinones or participate in Maillard-type reactions, thus limiting greening and promoting browning. However, the absence of thiols allows quinones to react with nucleophilic groups on proteins, leading to the formation of green TBA derivatives.

Chlorogenic Acid and Protein Content of Sunflower Flour Protein Powders

Table 2 shows the chlorogenic acid (CGA) concentrations and protein content of sunflower flour protein powders extracted at two alkaline pH levels (9 and 11) under varying concentrations of glutathione [GSH] and cysteine [Cys]. The protein content at pH 9 and 11 ranged from 78.20% to 84.80% and 73.10% to 78.02%, respectively (Table 2), with no statistically significant differences across treatments (p < 0.05), as confirmed by Tukey's Studentized Range Test. These results indicate that neither the type nor concentration of thiol compounds (GSH and Cys at 2.8 and 5.6 mM) altered protein concentration under the tested alkaline conditions. Although Salgado et al., (2011) did not include thiols in their extraction protocol, they similarly observed that variations in extraction parameters such as pH and solvent composition did not change the protein content of sunflower protein concentrates. Thus, while the extraction conditions differ, these studies consistently report that protein content remains stable across a range of extraction treatments, as in the present study. This is also comparable to the findings of Zhang et al., (2019), who observed structural changes in sunflower proteins without significant alterations to protein content.

Table 1

Chlorogenic Acid (mg/g) and protein content (%) of Sunflower flour protein powders

pH Level	Thiol Type and Concentration	Chlorogenic Acid Content (mg/g) M ± SD ^{Ns}	Protein Content (%) M ± SD ^{Ns}
pH 9	0.0 mM Thiols	0.28 ± 0.04	84.80 ± 7.27
	2.8 mM GSH	0.31 ± 0.12	79.59 ± 7.51
	5.6 mM GSH	0.23 ± 0.02	80.66 ± 8.40
	2.8 mM Cys	0.31 ± 0.03	84.06 ± 11.73
	5.6 mM Cys	0.29 ± 0.06	78.20 ± 6.96
pH 11	0.0 mM Thiols	0.13 ± 0.04	73.10 ± 13.45
	2.8 mM GSH	0.14 ± 0.06	74.32 ± 16.45
	5.6 mM GSH	0.17 ± 0.14	75.90 ± 14.75
	2.8 mM Cys	0.16 ± 0.08	78.02 ± 9.51
	5.6 mM Cys	0.19 ± 0.10	77.00 ± 7.69
C.V. %		17.55	13.75
P value		0.2778	0.4711

Note. GSH: Glutathione; Cys: Cysteine; C.V.: Coefficient of variation calculated as (SD/mean) × 100; P-value: Probability value: M: Mean; SD: Standard deviation; Ns: non-significant differences found.

The CGA content ranged from 0.13 ± 0.04 mg/g in the 0.0 mM treatment at pH 11 to 0.31 ± 0.12 mg/g in the 2.8 mM GSH treatment at pH 9. The highest CGA concentrations across all groups were observed at pH 9, regardless of thiol type or concentration, suggesting that milder alkaline conditions favor CGA retention; however, these results are conclusive since no-significant difference was found. Although the lowest CGA values appeared at pH 11 with GSH, these differences were not statistically significant. The general linear model (GLM) analysis showed no statistically significant difference in CGA content across the combined treatments of pH and thiol levels (p = 0.2778). Tukey's HSD test confirmed that all treatment means fell within the same statistical group, indicating no significant differences.

Higher pH was expected to promote thiol reactivity, which can lower soluble chlorogenic acid content and improve protein functionality by facilitating thiol interaction with electrophilic species like glucose intermediates and CGA quinones (Ali et al., 2024) . The observed trend of higher CGA values at pH 9 and lower at pH 11 is in line with the known pH sensitivity that CGA has (Table 2). CGA is prone to oxidation and quinone formation under strong alkaline conditions, especially in the absence of effective reducing agents (Liang & Were, 2020). Cysteine, with a lower pKa and greater nucleophilic reactivity, tends to form more stable conjugates with CGA quinones than glutathione, as observed in values tending to be higher than GSH, offering greater protection against degradation of chlorogenic acid content. (Ishii et al., 2021; Liang & Were, 2020).

Functional Properties of Sunflower Flour Protein Powders

Water holding Capacity (WHC) and Solubility

The highest WHC value of 1.90 ± 0.086 g/g was observed at pH 9 with 2.8 mM GSH), while the lowest was found at pH 11 without thiols at 1.03 ± 0.020 g/g (Table 3). Although WHC appeared slightly higher at pH 9, the differences were not statistically significant consistent with the findings of (Kaur & Ghoshal, 2022), who noted that WHC increases on both sides of the isoelectric point due to reduced protein–protein interactions and increased protein–water interactions. The pH of the sunflower protein powders was around 5-6, which is close to the isoelectric point (IEP) of helianthinin proteins, ranging from pH 5 to 6. At this pH, the proteins have minimal net charge, leading to aggregation and a reduction in both solubility and water-holding capacity (WHC). As the proteins aggregate, their interaction with water decreases, causing a drop in WHC and solubility. Outside this pH range, proteins unfold and improve their water-binding capacity and solubility.

At alkaline pH, proteins tend to unfold and expose more water-binding sites to enhance WHC, particularly in the presence of charged or hydrophilic amino acid residues such as amide, hydroxyl, and peptide groups capable of interacting with water molecules (Kaur & Ghoshal, 2022). . In contrast to expectations, the addition of thiol compounds did not significantly affect WHC. This suggests that any potential conformational changes induced by cysteine or glutathione at concentrations used were insufficient to alter the number or accessibility of water-binding sites in a statistically meaningful way. Interestingly, the addition of thiol compounds like cysteine or glutathione did not significantly alter WHC at either pH. This implies that any conformational changes due to the thiols were insufficient to modify the overall WHC under these conditions. As previously noted by

(Kaur and Ghoshal, 2022) and (Kinsella, 1979), WHC is primarily influenced by the protein's structure, charge distribution, and molecular expansion rather than by the presence of additives like thiols.

The nitrogen solubility index (NSI) of sunflower flour protein powders ranged from 0.89 ± 0.23% to 1.45 ± 0.77%, with no statistically significant differences (p = 0.4711) observed among treatments across varying pH levels and thiol concentrations (Table 3). These values are markedly lower than those reported in literature, where solubility levels of sunflower protein isolates typically exceed 70% and can reach up to 90% under optimized extraction conditions such as the use of ascorbic acid and N-acetylcysteine, combined with controlled pH and temperature settings (Ali et al., 2024; Salgado et al., 2011; Tantawy et al., 2025). Ali et al., (2024) for instance reported protein solubility above 90% in sunflower protein concentrates extracted with ascorbic acid and N-acetylcysteine (NAC), while (Tantawy et al., 2025) observed solubility levels ranging from 72.41% to above 90% when employing NAC and ascorbic acid as anti-greening agents. They measured protein content of supernatant/protein content of dispersion.

Similarly, (Salgado et al., 2012) noted sunflower protein concentrates and isolates with water solubility consistently higher than 75%.

Table 2

Water Holding Capacity and Nitrogen Solubility of Sunflower flour protein powders.

pH Level	Thiol Type and Concentration	Water Holding Capacity (g of water/g of dry pellet) M ± SD Ns	Nitrogen Solubility % M ± SD Ns
pH 9	0.0 mM Thiols	1.51 ± 0.09	0.89 ± 0.23
	2.8 mM GSH	1.90 ± 0.08	1.18 ± 0.21
	5.6 mM GSH	1.43 ± 0.07	1.10 ± 0.07
	2.8 mM Cys	1.34 ± 0.05	1.02 ± 0.17
	5.6 mM Cys	1.44 ± 0.06	1.16 ± 0.06
pH 11	0.0 mM Thiols	1.03 ± 0.02	1.10 ± 0.15
	2.8 mM GSH	1.44 ± 0.01	1.45 ± 0.07
	5.6 mM GSH	1.08 ± 0.01	1.08 ± 0.18
	2.8 mM Cys	1.04 ± 0.01	1.15 ± 0.25
	5.6 mM Cys	1.05 ± 0.02	1.15 ± 0.32
C.V. %		3.40	15.16
P value		0.5516	0.4711

Note. GSH: Glutathione; Cys: Cysteine; C.V.: Coefficient of variation calculated as (SD/mean) × 100; P-value: Probability value: M: Mean; SD: Standard deviation; Ns: non-significant differences found.

In the present study, thiol-based ingredients (glutathione and cysteine) did not significantly enhance solubility, which may reflect suboptimal extraction conditions, insufficient interaction time, or limited conformational modification of the proteins. This observation aligns with (Ali et al., 2024), who reported that the addition of thiols did not significantly change protein solubility in sunflower protein concentrates under similar conditions.

The considerably lower solubility values obtained in this study might be attributed to differences in extraction methods, calculations, and processing conditions. The pH affects solubility, with proteins typically showing lower solubility near their isoelectric point (around pH 5.6 for helianthinin), leading to aggregation and decreased solubility. In contrast, the present study employed less intensive extraction parameters, without extensive protein isolation steps, possibly resulting in less effective protein solubilization and greater protein aggregation or denaturation. Moreover, the presence of phenolic compounds, specifically chlorogenic acid (CGA), might further contribute to lowered protein solubility, CGA can interact covalently and non-covalently with proteins, potentially causing structural modifications and aggregation under alkaline conditions (González-Pérez, 2003; Jia et al., 2022). (González-Pérez, 2003;

Additionally, processing history like alkaline pH, isoelectric precipitation and ingredient composition may have also influenced solubility outcomes. For example, (Jia et al., 2022) demonstrated that the deoiling bymechanical cold pressing or solvent extraction (e.g., hexane), critically lowered subsequent functional properties, including nitrogen solubility. Hence, variations in processing history, such as oil extraction methods or ethanol washing, could have significantly impacted the observed solubility in this study. Therefore, the lower solubility values reported in this study compared to existing literature can be reasonably attributed to differences in extraction methodologies, phenolic content, and processing conditions. Alkaline extraction method affecting protein structure, the interaction of chlorogenic acid (CGA) with proteins reducing solubility, and the absence of additional treatments or temperature controls compared to other studies. These findings highlight the importance of optimizing extraction procedures and controlling phenolic interactions to improve the functional properties and overall applicability of sunflower protein products.

Oil Holding Capacity (OHC) and Hydrophobicity of Sunflower flour protein powders

The GLM analysis indicated no statistically significant effect ($p = 0.6996$) of pH, thiol type, or concentration on the oil-holding capacity (OHC) of sunflower protein isolates. A lack of significant differences between means suggests that thiol addition and pH changes did not meaningfully alter the oil retention behavior of the sunflower protein flours (Table 4). These findings aligned with the literature on oil holding capacity (OHC) and its relationship with protein structure. According to (Kaur & Ghoshal, 2022), OHC is closely linked to the exposure of hydrophobic amino acid residues, particularly those found in unfolded or partially denatured regions, as these residues interact with oil through non-polar interactions. This is reflected in the superior OHC observed in sunflower proteins derived from kernels rather than whole seeds, as reported by (Ali et al., 2024) and (Salgado et al., 2011).

The higher protein purity in kernel-derived proteins likely contributes to this enhanced OHC by minimizing interference from fiber and polyphenolic compounds, which may reduce the exposure of hydrophobic residues and thereby impact oil retention. Surface hydrophobicity values ranged from 18,200 So to 38,800 So, with no statistically significant differences across treatments ($p > 0.05$), suggesting that thiol addition and pH did not markedly alter protein surface properties. This stability may reflect limited unfolding or reorganization of hydrophobic regions, consistent with (Kinsella, 1979) view that hydrophobicity depends on protein structure and residue accessibility all of which appear largely unaffected by the treatments tested.

The lack of statistically significant differences in this study suggests that thiol-induced structural modifications under alkaline conditions were not sufficient to alter protein surface hydrophobicity or conformation to a degree that would impact oil binding capacity (Table 4). This aligns with (Kaur & Ghoshal, 2022) observations that thiol interactions may affect protein solubility and oxidative stability, but do not necessarily enhance oil-binding functionality unless accompanied by substantial unfolding or denaturation.

Table 3

Oil Holding Capacity (OHC) and Hydrophobicity of Sunflower flour protein powders

pH Level	Thiol Type and Concentration	OHC (g of oil retained/g of dry matter) M ± SD Ns	Hydrophobicity (So) M ± SD Ns
pH 9	0.0 mM Thiols	0.44 ± 0.03	34600.00 ± 2825.50
	2.8 mM GSH	0.42 ± 0.03	29600.00 ± 1697.79
	5.6 mM GSH	0.39 ± 0.07	38800.00 ± 3962.10
	2.8 mM Cys	0.45 ± 0.07	30900.00 ± 5949.53
	5.6 mM Cys	0.35 ± 0.03	34700.00 ± 1880.61
pH 11	0.0 mM Thiols	0.47 ± 0.03	18200.00 ± 4445.64
	2.8 mM GSH	0.48 ± 0.03	27100.00 ± 7022.40
	5.6 mM GSH	0.54 ± 0.04	23400.00 ± 3889.85
	2.8 mM Cys	0.48 ± 0.03	23000.00 ± 3756.16
	5.6 mM Cys	0.48 ± 0.04	19500.00 ± 1411.14
C.V. %		8.98	13.17
P -value		0.6996	0.3211

Note. GSH: Glutathione; Cys: Cysteine; C.V.: Coefficient of variation calculated as (SD/mean) × 100; P-value: Probability value: M: Mean; SD: Standard deviation; Ns: non-significant differences found.

Effect of thiol type, concentration, and pH on Maillard Reaction Products

Hydroxymethylfurfural (HMF)

The browning index (BI) results in Figure 2 showed that cysteine promoted the highest browning, with peak values at pH 11 + 2.8 mM cysteine (BI = 76.13) and pH 9 + 2.8 mM cysteine (BI = 72.21). However, the corresponding HMF levels were moderate, measuring 63.60 ± 15.00 and 40.43 ± 20.20 mg/g, respectively (Table 5). Similarly, the treatment with 5.6 mM cysteine at pH 9 produced a high BI (67.94) but only 54.58 ± 2.29 mg/g HMF.

In contrast, the highest HMF concentration (88.08 ± 28.45 mg/g) occurred in the pH 11 + 2.8 mM GSH treatment, which exhibited relatively low browning (BI = 23.46).

Overall, HMF contents across treatments were low, ranging from 40.43 ± 20.20 to 88.08 ± 28.45 mg/g, with no significant differences among treatments ($p = 0.5519$). This pattern indicates that HMF accumulation was not aligned with browning intensity and that cysteine-induced browning proceeded largely through alternative Maillard pigments.

The low HMF concentrations observed here are consistent with the aqueous and alkaline extraction conditions, which are not favorable for HMF generation. Formation of this compound is generally promoted by heating, low moisture, and acidic conditions (Capuano y Fogliano, 2011; Kroh, 1994). Under the mild, high-moisture environment of this study, HMF accumulation was limited, even when browning index values were high.

These results suggest that browning in sunflower protein powders is primarily associated with Maillard- and thiol–quinone-derived pigments rather than HMF. The findings are in line with (Ajandouz et al., 2008), who demonstrated that reaction pathways and end-products of the Maillard reaction are strongly dependent on pH and processing conditions, with alkaline and aqueous systems generally limiting HMF formation.

Table 4

Hydroxymethylfurfural (HMF) Content mg/g and Fluorescence of advanced Maillard products and soluble tryptophan (FAST) index of Sunflower flour protein powders of Sunflower flour protein powders.

pH Level	Thiol Type and Concentration (mM)	HMF (mg/g) M ± SD Ns	Fast Index (FI) M ± SD Ns
pH 9	0.0 mM Thiols	51.45 ± 11.40	156.60 ± 16.33
	2.8 mM GSH	65.73 ± 17.29	118.07 ± 14.63
	5.6 mM GSH	52.64 ± 5.01	189.05 ± 39.18
	2.8 mM Cys	40.43 ± 20.20	236.77 ± 47.16
	5.6 mM Cys	54.58 ± 2.29	313.90 ± 19.70
pH 11	0.0 mM Thiols	47.84 ± 28.44	141.29 ± 16.25
	2.8 mM GSH	88.08 ± 28.45	176.30 ± 11.27
	5.6 mM GSH	62.22 ± 10.70	322.19 ± 18.96
	2.8 mM Cys	63.60 ± 15.00	277.59 ± 18.31
	5.6 mM Cys	67.40 ± 13.49	329.47 ± 45.97
C.V. %		25.92	10.96
P value		0.5519	0.605

Note. GSH: Glutathione; Cys: Cysteine; C.V.: Coefficient of variation calculated as (SD/mean) × 100; P-value: Probability value: M: Mean; SD: Standard deviation; Ns: non-significant differences found.

The highest FAST index was recorded in powders treated with 5.6 mM cysteine at pH 11 (329.47 ± 45.97), followed closely by 5.6 mM GSH (322.19 ± 18.96) and 5.6 mM cysteine at pH 9 (313.90 ± 19.70). In contrast, the lowest value (118.07 ± 14.63) was observed in the powders with

2.8 mM GSH at pH 9. Despite these numerical values, no statistical significance was detected. The Fluorescence of advanced Maillard products and soluble tryptophan (FAST index) , which reflects the extent of Maillard-induced structural modifications relative to tryptophan fluorescence, revealed no statistically significant differences across treatments. These findings suggest that neither thiol type (glutathione or cysteine), concentration (2.8 or 5.6 mM), nor pH (9 or 11) produced measurable changes in the relationship between tryptophan degradation and fluorescent Maillard product formation under the current experimental conditions.

Advanced Fluorescent Glycation End Products (AGEs) of Sunflower flour protein powders

When compared with the browning index (BI), fluorescent AGEs formation did not follow the same trend. Cysteine treatments that produced the highest BI values (e.g., 2.8 mM Cys at pH 11 and pH 9) consistently showed lower AGE levels than controls or glutathione. This indicates that while cysteine enhanced browning, it simultaneously reduced AGE formation, suggesting that its reactivity diverted intermediates toward pigment formation rather than fluorescent AGEs. No statistically significant differences were observed in fluorescent advanced glycation end products (AGEs) after 4, 8, or 24 hour incubation at 50°C (Table 6). This demonstrates that thiol addition, whether cysteine or glutathione, did not significantly influence AGE formation under the tested conditions.

At 4 h, AGE values ranged from 34,495 (2.8 mM Cys at pH 11) to 43,196 (control at pH 9). Both cysteine- and glutathione-treated samples showed comparable values to the controls, but the lowest numerical AGE levels were observed in cysteine treatments, particularly 2.8 mM cysteine at pH 11. Glutathione treatments, by contrast, clustered closer to the control values, with the exception of 5.6 mM GSH at pH 9 (34,555), which also appeared lower than the control but not as low as cysteine at pH 11. Thus, while no statistical differences were detected, cysteine treatments tended to be associated with numerically reduced AGE concentrations at this early stage.

At 8 h, AGE levels again showed no statistical separation, spanning from 28,854 (2.8 mM Cys at pH 11) to 43,849 (5.6 mM GSH at pH 9). At 8 hours, powders with added cysteine again trended toward lower values (28,854–33,253) compared with glutathione treatments (36,052–43,849). This suggests that cysteine may have had a slightly higher limiting AGE accumulation under prolonged alkaline incubation, although the variability (CV = 7.27%) and groupings confirm the absence of significance.

At 24 h, values ranged from 31,747 (2.8 mM Cys at pH 11) to 47,325 (5.6 mM GSH at pH 9). Here too, cysteine treatments showed the lowest numerical values, whereas glutathione treatments and controls exhibited higher AGE levels. However, the variation among replicates (CV = 13.38%) was larger at this time point, further supporting the conclusion that none of the observed differences can be interpreted as statistically meaningful**.**

Taken together, these results confirm that neither cysteine nor glutathione significantly altered fluorescent AGE formation at any time point when incubated at 50 C. Nonetheless, the consistent numerical trend of lower AGE values in cysteine treatments suggests that cysteine may interact more efficiently with reactive dicarbonyl intermediates than glutathione, particularly under highly alkaline conditions. This is in line with previous reports by (Ishii et al., 2021) who observed that cysteine strongly influenced color and browning index but not fluorescent AGEs, and Liang & Were (2020) likewise noted that cysteine modulates carbonyl chemistry in sunflower protein systems without suppressing AGE formation. As reported by (Lim et al., 2010), cysteine can increase browning while reducing 3-deoxyglucosone (3-DG) levels—a key intermediate in AGE formation. This supports the idea that cysteine accelerates Maillard pigment formation by reacting early with reactive dicarbonyls, enhancing the browning index without proportionally raising fluorescent AGE levels.It is important to note that these measurements were conducted after incubation at 50 °C to accelerate AGE development. At room temperature, overall AGE formation would be expected to remain very low, but the same pattern of cysteine showing lower values than controls or glutathione would likely be even more evident due to the slower reaction rate under mild conditions.

Table 5

Advanced Fluorescent Glycation End Products (AGEs) of Sunflower flour protein powders incubated at 50 °C.

pH Level	Thiol Type and Concentration	4 h	8 h	24 h	C.V. %	P value
pH 9	0.0 mM Thiols	43196.00 ± 2900.47^{ax}	31978.83 ± 1638.01^{ax}	37798.83 ± 1094.18^{ax}	19.01	0.3465
	2.8 mM GSH	37912.17 ± 2390.09^{ax}	34120.17 ± 1399.85^{ax}	34015.00 ± 3316.37^{ax}	32.61	0.8965
	5.6 mM GSH	34555.17 ± 2914.96^{ax}	43849.50 ± 3862.77^{ax}	47325.67 ± 3747.30^{ax}	8.00	0.5318
	2.8 mM Cys	39115.67 ± 1522.38^{ax}	33253.00 ± 2472.75^{ax}	36465.67 ± 2263.23^{ax}	14.31	0.4351
	5.6 mM Cys	35134.33 ± 2368.34^{ax}	33004.67 ± 2114.01^{ax}	35959.83 ± 14045.68^{ax}	28.99	0.9341
pH 11	0.0 mM Thiols	35614.33 ± 3659.02^{ax}	31116.17 ± 2776.08^{ax}	33747.17 ± 2324.37^{ax}	21.52	0.7548
	2.8 mM GSH	40233.50 ± 2482.74^{ax}	36052.50 ± 2170.25^{ax}	35837.50 ± 1053.89^{ax}	22.82	0.7841
	5.6 mM GSH	38410.33 ± 3617.33^{ax}	36850.33 ± 3345.95^{ax}	35725.00 ± 9665.10^{ax}	18.59	0.8930
	2.8 mM Cys	34495.50 ± 2206.40^{ax}	28854.33 ± 3917.68^{ax}	31747.67 ± 9973.17^{ax}	26.86	0.7317
	5.6 mM Cys	36539.33 ± 1650.80^{ax}	34339.33 ± 1285.24^{ax}	31878.50 ± 1550.41^{ax}	31.55	0.8719
	C.V. %	6.85	7.27	13.38		
	P value	0.3802	0.6873	0.8133		

Note. GSH: Glutathione; Cys: Cysteine; C.V.: Coefficient of variation calculated as (SD/mean) × 100; P-value: Probability value; M: Mean; SD: Standard deviation; means by column with the same letter "a" are not significantly different for treatments. Means by row with the same letter "x" are not significantly different over time.

Conclusions

Thiol addition significantly influenced the color characteristics of sunflower protein isolates. Cysteine´s treatments showed the most effective greening mitigation and the highest browning intensity. All thiol treatments lowered greening compared to controls, with cysteine outperforming glutathione. This confirms cysteine's superior reactivity with CGA quinones, limiting green pigment formation under alkaline conditions.

Thiol addition did not affect the functional properties of sunflower protein (water/oil-holding capacity, solubility, and hydrophobicity remained unchanged).

Protein functionality was preserved across all treatments, suggesting that thiol compounds do not compromise structural integrity under the tested extraction conditions.

Maillard reaction products showed limited formation, with HMF, FAST index, and fluorescent AGE levels unchanged across treatments. Cysteine consistently trended toward lower AGE values, suggesting some early carbonyl scavenging, while, glutathione and controls remained higher. Overall, thiols influenced browning more than fluorescent AGE accumulation.

Recommendations

The use of cysteine, particularly at 5.6 mM and pH 11, is recommended for industrial extraction of sunflower protein isolates to mitigate greening and enhance visual quality without affecting protein functionality.

Further studies should evaluate the impact of thiol treatments on sensory properties and consumer acceptance in final food products, especially those incorporating sunflower protein as a visible ingredient.

References

Ajandouz, E., Desseaux, V., Tazi, S. Y Puigserver, A. (2008). Effects of temperature and pH on the kinetics of caramelisation, protein cross-linking and Maillard reactions in aqueous model systems. *Food Chemistry*, *107*(3), 1244–1252. https://doi.org/10.1016/j.foodchem.2007.09.062

Ali, M., Schmidt, C., Walczak, M., Förster, A., Sayed, A., Khalil, M., Rizk, A., Aly, S. y Hellwig, M. (2024). Improvement of protein extraction from sunflower oil cake using ascorbic acid and N-acetylcysteine: Effects on chemical, structural, and functional properties. *Journal of Food Measurement and Characterization*, *18*(8), 7198–7212. https://doi.org/10.1007/s11694-024-02730-4

Capuano, E. y Fogliano, V. (2011). Acrylamide and 5-hydroxymethylfurfural (HMF): A review on metabolism, toxicity, occurrence in food and mitigation strategies. *LWT*, *44*(4), 793–810. https://doi.org/10.1016/j.lwt.2010.11.002

Favre, L. C., Rolandelli, G., Mshicileli, N., Vhangani, L. N., Dos Santos Ferreira, C., van Wyk, J. y Del Buera, M. P. (2020). Antioxidant and anti-glycation potential of green pepper (Piper nigrum): Optimization of β-cyclodextrin-based extraction by response surface methodology. *Food Chemistry*, *316*, 126280. https://doi.org/10.1016/j.foodchem.2020.126280

González-Pérez, S. (2003). Physico-chemical and functional properties of sunflower proteins. Physico-chemical and functional properties of sunflower proteins /

Ishii, A. K., Toto Pacioles, C. y Were, L. (2021). Color and structural modifications of alkaline extracted sunflower protein concentrates and isolates using L-cysteine and glutathione. *Food Research International (Ottawa, Ont.)*, *147*, 110574. https://doi.org/10.1016/j.foodres.2021.110574

Jia, W., Sutanto, I. R., Ndiaye, M., Keppler, J. K. y van der Goot, A. J [Atze Jan] (2022). Effect of aqueous ethanol washing on functional properties of sunflower materials for meat analogue application. *Food Structure*, *33*, 100274. https://doi.org/10.1016/j.foostr.2022.100274

Karefyllakis, D., Salakou, S., Bitter, J. H., van der Goot, A. J [Atze J.] y Nikiforidis, C. V. (2018). Covalent Bonding of Chlorogenic Acid Induces Structural Modifications on Sunflower Proteins. *Chemphyschem : A European Journal of Chemical Physics and Physical Chemistry*, *19*(4), 459–468. https://doi.org/10.1002/cphc.201701054

Kaur, R. y Ghoshal, G. (2022). Sunflower protein isolates-composition, extraction and functional properties. *Advances in Colloid and Interface Science*, *306*, 102725. https://doi.org/10.1016/j.cis.2022.102725

Kinsella, J. E. (1979). Functional properties of soy proteins. *Journal of the American Oil Chemists' Society*, *56*(3Part1), 242–258. https://doi.org/10.1007/BF02671468

Kroh, L. W. (1994). Caramelisation in food and beverages. *Food Chemistry*, *51*(4), 373–379. https://doi.org/10.1016/0308-8146(94)90188-0

Liang, Y. y Were, L. (2020). Cysteine's effects on chlorogenic acid quinone induced greening and browning: Mechanism and effect on antioxidant reducing capacity. *Food Chemistry*, *309*, 125697. https://doi.org/10.1016/j.foodchem.2019.125697

Lim, S.-I., Kwak, E.-J., Lee, O.-H. y Lee, B.-Y. (2010). Effect of antibrowning agents on browning and intermediate formation in the glucose-glutamic acid model. *Journal of Food Science*, *75*(8), C678-83. https://doi.org/10.1111/j.1750-3841.2010.01797.x

Lund, M. N [Marianne N.] y Ray, C. A. (2017). Control of Maillard Reactions in Foods: Strategies and Chemical Mechanisms. *Journal of Agricultural and Food Chemistry*, *65*(23), 4537–4552. https://doi.org/10.1021/acs.jafc.7b00882

Nielsen, S. S. (2010). Protein Nitrogen Determination. En S. S. Nielsen (Ed.), *Food Science Texts Series. Food Analysis Laboratory Manual* (pp. 39–45). Springer US. https://doi.org/10.1007/978-1-4419-1463-7_5

Pepra-Ameyaw, N. B., Lo Verde, C., Drucker, C. T., Owens, C. P. y Senger, L. W. (2023). Preventing chlorogenic acid quinone-induced greening in sunflower cookies by chlorogenic acid esterase and thiol-based dough conditioners. *LWT*, *174*, 114392. https://doi.org/10.1016/j.lwt.2022.114392

Pickardt, C., Weisz, G. M., Eisner, P., Kammerer, D. R., Neidhart, S. y Carle, R. (2011). Processing of low polyphenol protein isolates from residues of sunflower seed oil production. *Procedia Food Science*, *1*, 1417–1424. https://doi.org/10.1016/j.profoo.2011.09.210

Pucci, M., Akıllıoğlu, H. G., Bevilacqua, M., Abate, G. y Lund, M. N [Marianne Nissen] (2024). Investigation of Maillard reaction products in plant-based milk alternatives. *Food Research International (Ottawa, Ont.)*, *198*, 115418. https://doi.org/10.1016/j.foodres.2024.115418

Salgado, P. R., Molina Ortiz, S. E., Petruccelli, S. y Mauri, A. N. (2011). Sunflower Protein Concentrates and Isolates Prepared from Oil Cakes Have High Water Solubility and Antioxidant Capacity. *Journal of the American Oil Chemists' Society*, *88*(3), 351–360. https://doi.org/10.1007/s11746-010-1673-z

Salgado, P. R., Molina Ortiz, S. E., Petruccelli, S. y Mauri, A. N. (2012). Functional Food Ingredients Based on Sunflower Protein Concentrates Naturally Enriched with Antioxidant Phenolic Compounds. *Journal of the American Oil Chemists' Society*, *89*(5), 825–836. https://doi.org/10.1007/s11746-011-1982-x

Sara, A. S., Mathé, C., Basselin, M., Fournier, F., Aymes, A., Bianeis, M., Galet, O. y Kapel, R. (2020). Optimization of sunflower albumin extraction from oleaginous meal and characterization of their structure and properties. *Food Hydrocolloids*, *99*, 105335. https://doi.org/10.1016/j.foodhyd.2019.105335

Sosulski, F. W. Y Mccurdy, A. (1987). Functionality of Flours, Protein Fractions and Isolates from Field Peas and Faba Bean. *Journal of Food Science*, *52*(4), 1010–1014. https://doi.org/10.1111/j.1365-2621.1987.tb14263.x

Tantawy, A. A., Ali, M., Kurbonova, M., El-Messery, T. M., Baranenko, D. A., Hellwig, M., Abozed, S. S., El-Said, M. M., Salama, M. A., Abdin, M., Rizk, A., Khalil, M., Eid, M. H., Saeed, O., Mahmoud, S. F. y Aly, S. (2025). Functional properties of sunflower protein concentrates extracted using different anti-greening agents- low-fat whipping cream preparation. *LWT*, *218*, 117456. https://doi.org/10.1016/j.lwt.2025.117456

Wildermuth, S. R., Young, E. E. y Were, L. M. (2016). Chlorogenic Acid Oxidation and Its Reaction with Sunflower Proteins to Form Green-Colored Complexes. *Comprehensive Reviews in Food Science and Food Safety*, *15*(5), 829–843. https://doi.org/10.1111/1541-4337.12213

Zhang, W., Liu, Y., Hu, M. y Yang, R. (2019). Preparation of high-quality sunflower seed protein with a new chlorogenic acid hydrolase from Aspergillus niger. *Biotechnology Letters*, *41*(4-5), 565–574. https://doi.org/10.1007/s10529-019-02654-w

Zhang, Y. y Xiao, Z. (2025). Surface Hydrophobicity. En Y. Li (Ed.), *Methods and Protocols in Food Science. Plant-Based Proteins* (pp. 279–286). Springer US. https://doi.org/10.1007/978-1-0716-4272-6_21

Zieliński, H., Amigo-Benavent, M., Castillo, M., Michalska-Ciechanowska, A. y Zielinska, D. (2010). Formulation and baking process affect Maillard reaction development and antioxidant capacity of ginger cakes. *Journal of Food and Nutrition Research*, *49*, 140–148.