Production and Functional Properties of Protein Isolate from Sunflower Meal

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Abstract – Alkaline extraction and precipitation were used to obtain protein isolate from sunflower meal. The isolate yield was 68.4%, protein content was 91.5%, and maximum solubility reached 88.2% at pH 8. These results support further evaluation of sunflower meal as a source of functional protein ingredients.

Keywords – sunflower meal, protein isolate, alkaline extraction, protein precipitation, protein content, solubility, food ingredients.

Introduction

Sunflower meal (*Helianthus annuus* L.) is the main by-product of sunflower oil production. It is an accessible and inexpensive source of high-quality plant protein. Sunflower protein is distinguished by a balanced amino acid composition with a high content of sulfur-containing amino acids (methionine and cysteine) and low allergenicity (compared with soybean), which makes it a very attractive component for modern food formulations.

However, the effective use of sunflower meal in the food industry is complicated by a number of technological problems. During industrial oil extraction, intensive mechanical pressing and heat treatment cause partial protein denaturation and the formation of intermolecular cross-links, which significantly reduces protein extractability. In addition, sunflower seeds contain a considerable amount of phenolic compounds, predominantly chlorogenic acid (up to 4% of dry weight) and caffeic acid. Under alkaline extraction conditions, these polyphenols readily oxidize and form covalent complexes with the amino groups of proteins. As a result of this interaction, the protein isolate acquires an undesirable dark green or brown coloration, while its solubility, enzymatic digestibility, and functional characteristics also deteriorate [1], [2].

For maximum protein extraction while preserving its valuable functional properties, controlled alkaline extraction followed by isoelectric precipitation remains one of the most practical methods suitable for industrial scale-up. The addition of reducing agents permitted for use in the food industry, for example, Na_2SO_3, during alkaline extraction makes it possible to effectively inhibit polyphenol oxidation and prevent the formation of protein–polyphenol complexes, thereby contributing to increased isolate purity and improved color.

The aim of this work was to investigate the alkaline extraction process for obtaining a high-purity protein isolate from sunflower press cake, analyze its chemical composition, and evaluate its functional properties, in particular solubility, emulsifying activity, and foaming capacity as a function of pH, with a view to further use in the food industry.

Materials and Methods

Sunflower press cake was obtained from a local vegetable oil production facility, ground in a laboratory mill, and defatted with toluene. All chemical reagents used in this study, including sodium hydroxide (NaOH), hydrochloric acid (HCl), sodium sulfite (Na2SO3), and bovine serum albumin (BSA), were of analytical grade (Sigma-Aldrich, USA).

Before extraction, the defatted sunflower meal was sieved to a uniform particle size of less than 0.5 mm. Alkaline protein extraction was carried out at a solid-to-liquid ratio of 1:10 (weight/volume). The aqueous suspension was adjusted to pH 9.5 using 1.0 M NaOH and incubated at 45 °C for 60 minutes under continuous mechanical stirring at 300 rpm. To reduce polyphenol oxidation, sodium sulfite (0.1% w/v) was introduced into the extraction medium.

After extraction, the suspension was centrifuged at 4000 rpm for 20 minutes at 4°C to separate the insoluble cell wall material. The clear supernatant containing soluble proteins was collected, and the pH was adjusted to 4.5 using 1.0 M HCl to induce isoelectric precipitation of the predominant globulin protein fraction. The resulting protein complex was isolated by centrifugation at 5000 rpm for 15 minutes, washed twice with distilled water adjusted to pH 4.5, neutralized to pH 7.0 with 0.1 M NaOH, freeze-dried, and stored at -20°C until further analysis. The production process is summarized in Fig. 1.

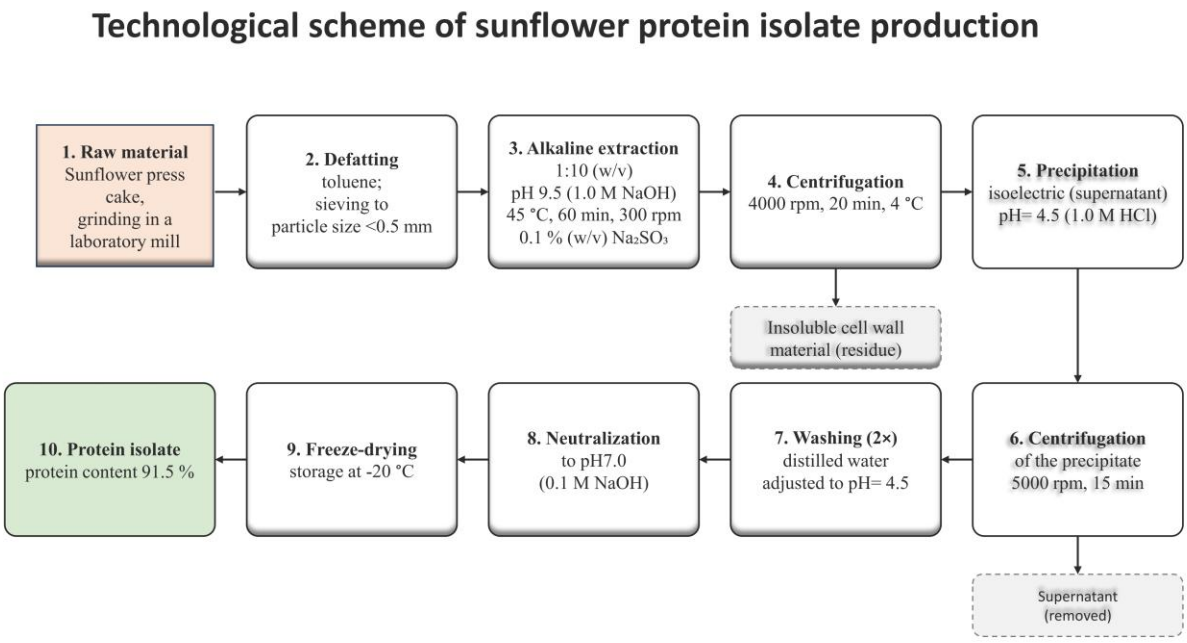

Fig. 1. Technological scheme of sunflower protein isolate production

Protein content was determined by the standard Kjeldahl method. Moisture, fat, and ash contents were evaluated gravimetrically using standard procedures.

Protein solubility was determined by preparing a 1.0% (w/v) protein dispersion in distilled water. The pH was adjusted using 0.1 M HCl or 0.1 M NaOH, and the suspensions were stirred for 30 min at room temperature. The mixtures were then centrifuged at 10000 rpm for 15 minutes, and the protein concentration in the supernatant was measured by the Bradford assay using BSA as a standard. Solubility was expressed as the percentage of soluble protein relative to the total protein content.

The emulsifying activity index (EAI) and emulsion stability index (ESI) were determined by homogenizing 15 mL of a 1.0% (w/v) protein solution at different pH values with 5 mL of refined sunflower oil using a high-shear homogenizer at 12 000 rpm for 2 min.

Foam capacity (FC) and foam stability (FS) were evaluated by whipping 20 mL of a 2.0% (w/v) protein dispersion at 10 000 rpm for 2 min. Foam volume was measured immediately after whipping to determine FC (%) and after standing for 30 min at room temperature to evaluate FS (%). All experiments were performed in triplicate, and the data are presented as mean ± standard deviation.

Results and Discussion

The initial protein content of the defatted sunflower press cake was (38.2 ± 0.8)% on a dry matter basis. Under the selected extraction conditions (pH 9.5; 45 °C; 60 min; 0.1% Na2SO3), the protein isolate yield was (68.4 ± 1.2)%, and the protein content of the dry isolate reached (91.5 ± 0.5)%, which corresponds to the industrial standard for protein isolates (protein content > 90%). The chemical composition of the initial sunflower meal and the resulting isolate is presented in Table 1.

Table 1

Chemical composition of sunflower press cake and protein isolate

Component	Sunflower meal, %	Sunflower protein isolate, %
Protein	38.2 ± 0.8	91.5 ± 0.5
Moisture	8.5 ± 0.3	4.2 ± 0.2
Fat	1.8 ± 0.1	0.6 ± 0.1
Ash	6.4 ± 0.2	2.1 ± 0.1

The low contents of residual lipids (0.6%) and ash (2.1%) in the isolate indicate the high effectiveness of the water-washing steps during the isolation process. A low lipid content is particularly important for increasing the stability and extending the shelf life of the protein ingredient.

The determination of solubility is also an important functional characteristic and affects emulsifying, foaming, and gel-forming capacity. The solubility profile of sunflower protein isolate over the pH range from 2.0 to 10.0 has a characteristic U-shaped curve typical of the storage globulin proteins of oilseed crops.

The minimum solubility (14.2%) was observed at pH 4.5, which corresponds to the isoelectric point (pI) of sunflower globulins (helianthinin). At this pH value, the net surface charge of the protein molecules approaches zero, which minimizes electrostatic repulsion and promotes hydrophobic aggregation and protein precipitation. As the pH deviates from the isoelectric point toward the acidic or alkaline range, protein solubility increases significantly as a result of an increase in the net negative or positive charge, which creates strong electrostatic repulsive forces between protein subunits.

At pH 2.0, solubility reached 64.5%. In neutral and alkaline media, solubility increased rapidly, reaching 76.8% at pH 7.0 and a maximum of 88.2% at pH 8.0–9.0. The high protein solubility in neutral and mildly alkaline media indicates considerable potential for the use of sunflower protein isolate in liquid food systems, protein beverages, and meat product analogues. The dependence of protein solubility on pH is shown in Fig. 2.

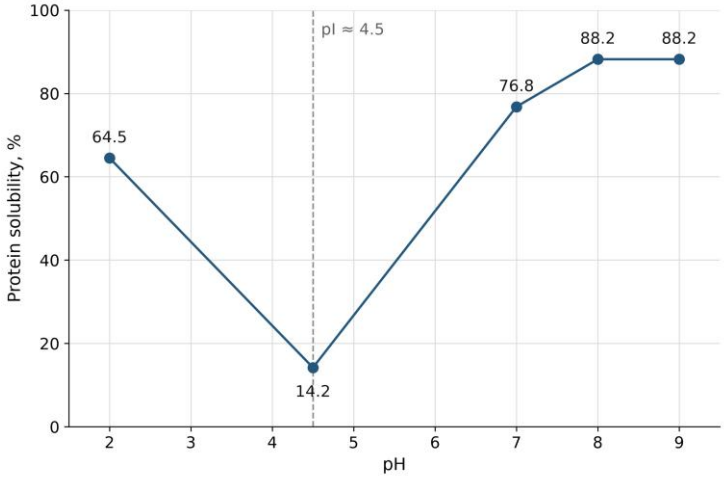

Fig. 2. Dependence of protein solubility on pH

The pH-dependent functional characteristics of the isolate, namely the emulsifying activity index (EAI), emulsion stability index (ESI), foaming capacity (FC), and foam stability (FS), are presented in Table 2.

Table 2

Functional characteristics of sunflower protein isolate at different pH values

pH	Solubility,%	EAI, m^2/g	ESI, min	FC, %	FS, %
2.0	64.5 ± 1.5	38.2 ± 1.1	22.4 ± 0.8	45.0 ± 2.0	30.0 ± 1.5
4.5	14.2 ± 0.8	12.1 ± 0.5	8.5 ± 0.4	15.0 ± 1.0	5.0 ± 0.5
7.0	76.8 ± 1.2	56.4 ± 1.4	38.6 ± 1.2	75.0 ± 3.0	58.0 ± 2.2
8.0	88.2 ± 1.0	68.9 ± 1.8	45.2 ± 1.5	92.0 ± 3.5	70.0 ± 2.5

The emulsifying activity of the protein isolate demonstrated a direct correlation with its solubility. At pH 8.0, when solubility was highest, the emulsifying activity index (EAI) reached a maximum value of (68.9 ± 1.8) m^2/g, and emulsion stability (ESI) was 45.2 min. In contrast, near the isoelectric point (pH 4.5), low solubility significantly limited the migration and orientation of proteins at the oil–water interface, resulting in low EAI (12.1 m^2/g) and ESI (8.5 min) values. Soluble protein molecules rapidly adsorb at the oil–water interface and form a continuous film that prevents droplet coalescence.

Similarly, foaming capacity and foam stability depended significantly on pH: the minimum values were observed at pH 4.5 (foaming capacity of 15%, foam stability of 5%), and the maximum values at pH 8.0 (92% and 70%, respectively). High solubility in an alkaline medium promotes the rapid coating of air bubbles during intensive whipping, while strong intermolecular interactions provide rigidity to the films in the foam, preventing their destruction.

To expand the possibilities for using sunflower protein isolate in acidic food products, for example, fruit juices, acidic beverages, or salad dressings with a pH of 3.5–4.5, limited enzymatic hydrolysis using food-grade endoproteases can be applied. This method is an effective approach to protein modification, as previously noted by Beaubier et al. [2].

Conclusion

The use of alkaline extraction (pH 9.5, 45°C, 60 min) with 0.1% Na2SO3 followed by isoelectric precipitation (pH 4.5) made it possible to successfully obtain a high-purity protein isolate from sunflower press cake. The overall product yield was 68.4%, and the crude protein content was 91.5% on a dry matter basis. The resulting isolate demonstrated exceptional solubility (88.2%), as well as good emulsifying (68.9 m^2/g) and foaming (92%) properties at pH 7.0–8.0. These results indicate that sunflower press cake protein isolate is a highly promising, environmentally sustainable functional ingredient for food products with a neutral pH, emulsified food products, and plant-based alternatives to animal proteins.

References

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