Sustainable Protein Extraction Technologies for the Valorization and Enhancement of a Sunflower Meal By-Product as an Alternative Protein Ingredient

By

Zainab Husain

A Thesis submitted to the

Faculty of Graduate and Postdoctoral Studies

of the University of Manitoba

in partial fulfillment of the requirements of the degree of

Master of Science

Department of Food and Human Nutritional Sciences

University of Manitoba

Winnipeg, Canada

Abstract

The continuous rise in global protein demand has increased interest in sustainable plant-based protein ingredients that can support future food production while reducing environmental impacts. Sunflower meal (SFM), a by-product of oil processing, contains approximately 30–50% protein on a dry-weight basis and remains underutilized. Conventional alkaline-based extraction can limit the food application of sunflower protein isolates (SFPI) by promoting protein denaturation, aggregation, and chlorogenic acid (CGA) oxidation, producing an undesirable dark green colour. This study investigated divalent salt-based, monovalent salt-based, and deep eutectic solvent (DES) extractions as sustainable alternatives for producing high-quality SFPI from commercial SFM. Alternative methods produced significantly higher protein purities, with MV-SFPI, DV-SFPI, and DES-SFPI containing 90.18%, 90.06%, and 88.46% protein, respectively, compared with 69.35% for ALK-SFPI. DES-SFPI achieved the highest extraction yield and protein recovery rate of 56.92% and 24.73%, respectively. Structural analysis showed greater aggregation and rearrangement in ALK-SFPI. DV-SFPI displayed the lightest off-white colour with an L* value of 84.07, while ALK-SFPI developed the darkest colour and a green hue. Techno-functional properties were influenced by extraction method and pH. MV-SFPI had the highest solubility at pH 7, DES-SFPI had the highest oil-holding capacity, and DV-SFPI showed the greatest foaming capacity under acidic and neutral conditions. Protein quality was further assessed through structural properties, amino acid composition, in vitro protein digestibility (IVPD), IV-PDCAAS, and anti-nutritional factors. All isolates retained characteristic 11S helianthinin and 2S albumin fractions but showed extraction-induced changes in secondary and tertiary structures. Amino acid profiles were comparable and rich in sulfur-containing amino acids, with lysine as the first limiting amino acid. IVPD remained high at 95.36%–98.75%. DV-SFPI had the highest IV-PDCAAS

(62.91%), compared with 50.34% for ALK-SFPI. DES-SFPI had the lowest total phenolic content (5.30 mg GAE/g dry weight), while ALK-SFPI had the lowest trypsin inhibitory activity (0.045 TUI/mg DW). Overall, salt-based and DES extractions effectively valorized commercial SFM into high-quality SFPI with improved protein purity, recovery, extraction yield, colour, nutritional quality, and comparable or enhanced techno-functionality. These findings support SFPI as a sustainable alternative to traditional animal protein sources while promoting waste reduction and a circular economy.

Acknowledgement

I would like to express my sincere appreciation to my supervisor, Dr. Nandika Bandara, for his guidance, encouragement, and continued support throughout my M.Sc. journey. His expertise, thoughtful direction, and mentorship were instrumental in the success of my research and professional development. I am especially grateful for the many opportunities I gained under his mentorship, which pushed me to grow as a researcher.

I would also like to thank my advisory committee members, Alphonsus Utioh and Dr. Michael Eskin, for their support, understanding, and valuable contributions to my work. Their encouragement and constructive feedback helped strengthen this thesis and played an important role in the successful completion of my degree.

I am deeply thankful for my lab members, whose friendship was an integral support system throughout this journey. Thank you for generously sharing your knowledge, training, and offering reassurance whenever hurdles arose. I would also like to thank John Bachu and Finn Makila for their support with experimentation and training.

To my husband, Ilyas, goes my most heartfelt gratitude. His love, patience, understanding, and unwavering belief in me formed the foundation that carried me through this journey. Thank you for always standing by my side during the happiest moments and the most difficult times. This accomplishment would not have been possible without you.

بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

ٱقۡرَأۡ بِٱسۡمِ رَبِّكَ ٱلَّذِى خَلَقَ ﴿١﴾

خَلَقَ ٱلۡإِنسَٰنَ مِنۡ عَلَقٍ ﴿٢﴾

ٱقۡرَأۡ وَرَبُّكَ ٱلۡأَكۡرَمُ ﴿٣﴾

ٱلَّذِى عَلَّمَ بِٱلۡقَلَمِ ﴿٤﴾

عَلَّمَ ٱلۡإِنسَٰنَ مَا لَمۡ يَعۡلَمۡ ﴿٥﴾

Table of Contents

List of Tables

List of Figures

List of Abbreviations

SFM: Sunflower Meal

SFPI: Sunflower Protein Isolates

CGA: Chlorogenic Acid

DES: Deep Eutectic Solvent

DES-SFPI: Deep Eutectic Solvent Extracted Sunflower Protein Isolates

DV-SFPI: Divalent Salt Extracted Sunflower Protein Isolates

MV-SFPI: Monovalent Salt Extracted Sunflower Protein Isolates

ALK-SFPI: Alkaline Extracted Sunflower Protein Isolates

SEM: Scanning Electron Microscopy

WHC: Water Holding Capacity

OHC: Oil Holding Capacity

EAI: Emulsification Activity Index

ESI: Emulsification Stability Index

LGC: Least Gelation Concentration

AAS: Amino Acid Score

TIA: Trypsin Inhibitor Activity

IVPD: In Vitro Protein Digestibility

IV-PDCAAS: In Vitro Protein Digestibility Corrected Amino Acid Score

Chapter 1: Introduction

The continuous rise in protein ingredients demand is attributed to various factors, including the growth of the global population which is expected to reach 9.7 billion by the year 2050; socio-economic changes such as increased household incomes and urbanization; and the recognition of proteins' role in healthy aging (Henchion et al., 2017; Smith et al., 2024; United Nations Department of Economic and Social Affairs, 2024). Feeding the future global population by expanding animal-based protein production creates several sustainability challenges, including substantial greenhouse gas emissions and excessive demands on land and freshwater for livestock rearing and feed (Henchion et al., 2017; Ismail et al., 2020). In response to these challenges, interest in plant-based proteins is growing among both consumers and the food industry, as they offer a way to support future protein needs while reducing environmental impacts.

Nearly 60% of plant protein sources are being used to produce animal feed, resulting in many indispensable sources that can otherwise be used for meeting human protein demand (Salter & Lopez-Viso, 2021). One such example is sunflower protein which is underutilized in the industry due to the limited research for optimization of novel extraction technologies to produce it as a viable and high-quality protein ingredient. Raw sunflower seeds contain 10–20% protein, 34–55% fat, 18–26% carbohydrates, 1.1–4.5% chlorogenic acid (CGA), and 2–4% total ash (Kaur & Ghoshal, 2022; Ren et al., 2015). Oilseeds such as sunflower are mainly used for oil extraction by pressing or chemical solvents due to their high fat content, which results in a sunflower meal (SFM) high in protein and fibre (Kaur & Ghoshal, 2022). Despite the challenges associated with using SFM as a protein source, it has promising potential due to its high protein content of 30–50% on a dry basis and provides a sustainable approach for utilizing a by-product to produce a protein ingredient (Ivanova et al., 2012; Ren et al., 2015). Compared to other oilseeds, sunflower

meal is widely available, high in protein, has low cost, and does not contain anti-nutritional factors such as protease inhibitors, cyanogens, and glucosinolates (Kaur & Ghoshal, 2022; Ren et al., 2015). Furthermore, sunflower proteins are highly digestible and have a high content of sulfur amino acids (methionine and cysteine) which are limiting in other plant protein sources.

Alkaline-based extraction is a conventional method for protein isolation and remains the industrial standard; however, it is associated with several limitations. This method is often associated with low extraction yield and reduced protein recovery, as well as harsh processing conditions that can negatively affect the nutritional quality and techno-functionality of the resulting protein ingredients. In addition, this process requires considerable solvent, water, and energy inputs, which limits its sustainability for large-scale industrial production (Hewage et al., 2022; Parodi et al., 2023). Alkaline-based extraction can be particularly challenging for producing SFPI, as high pH conditions used during extraction can promote undesirable reactions between reactive sunflower protein functional groups and CGA, which result in reduced techno-functionality, altered protein structure, and poor colour for food applications (Kaur & Ghoshal, 2022; Salgado et al., 2011).

On the other hand, salt-based and deep eutectic solvent (DES) extractions are more sustainable than alkaline-based extraction and provide improved protein yield and functional properties due to the less harsh conditions used (Aldalur et al., 2023; Hewage et al., 2022; Nadeeshani et al., 2026). Salt-based extraction is a conventional yet underutilized method for extracting plant-based proteins, based on the salting out or salting in phenomenon, in which the ionic strength of the protein is increased, thereby strengthening the interactions between the protein and salt, solubilizing and isolating it in water from insoluble non-protein materials. Furthermore, 60–80% of sunflower proteins are composed of helianthinin, an 11S globulin fraction that is salt-

soluble and can be extracted primarily by salt-based extraction (Ivanova et al., 2012; Kaur & Ghoshal, 2022). DES extraction is a novel green method that has been gaining interest in the food industry over the past couple of years. DES are generally low cost, sustainable, stable, non-toxic, and non-volatile mixtures of Lewis or Bronsted acids or bases (hydrogen donor and hydrogen acceptor) that can be used in food applications (Hewage et al., 2022).

Research on sunflower protein remains limited; thus, this study bridges this gap in the food industry by investigating novel and sustainable plant-based protein extraction technologies for SFPI production from SFM. As a by-product of the oil extraction industry, SFM is an underutilized protein-rich resource that can be valorized into high-quality SFPI, thereby supporting waste reduction and promoting a circular economy. Investigating SFPI as a protein source and functional ingredient is essential for expanding its applications in food systems, supporting future protein demand, and mitigating the environmental impacts associated with conventional protein production. In this context, green extraction approaches, such as salt-based and DES extractions, offer innovative alternatives that may improve extraction yield, protein purity, sensory properties, and tecno-functional performance, while limiting structural changes, thus enhancing the suitability of SFPI for diverse food applications.

Sunflower is also particularly relevance in the Canadian plant-protein sector, as it is a major oilseed crop widely produced in Canada. According to Statistics Canada (2024), Canadian sunflower production increased by 10% from 2022 to 2023, reaching 92,468 metric tons. Manitoba is especially important in this context, as its climate and soil conditions are well suited for sunflower cultivation, accounting for 93% of Canada's total sunflower production. Therefore, investigating sustainable extraction methods for producing high-quality SFPI from SFM is highly

relevant to advancing the future of both the Canadian and Manitoban plant-based protein industries.

1.1. Hypothesis

The sustainable extraction technologies, including monovalent salt-based, divalent salt-based, and DES extractions, will produce SFPI from SFM with higher protein purity, extraction yield, and protein recovery than alkaline-based extraction. Moreover, improved techno-functional properties, nutritional quality, and digestibility are expected due to the milder conditions associated with green methods, which limit the adverse effects of high pH and harsh conditions.

1.2. Objectives

1.2.1. Research Study 1

The objective of the first research study is to evaluate the potential of commercial SFM as a sustainable source for producing food-grade SFPI. Specifically, this study investigates the potential of divalent salt-based, monovalent salt-based, and DES protein extractions to replace the industry-standard alkaline-based extraction. SFPI samples were compared for protein purity, extraction yield, protein recovery, structural characteristics, sensory attributes, and physicochemical properties. The study also assessed how each extraction method affected the techno-functional properties of the resulting SFPI; therefore, determining their suitability for future food applications.

1.2.2. Research Study 2

The second research study aimed to determine how the three sustainable extraction technologies (divalent salt-based, monovalent salt-based, and DES protein extractions), compared with alkaline-based extraction, affect the nutritional quality and digestibility of SFPI. This study

compared SFPI samples by evaluating their protein composition, amino acid profile, amino acid score, in vitro protein digestibility, and digestibility-corrected amino acid score. In addition, the study examined antinutritional factors, including total phenolic content and trypsin inhibitory activity, to assess the potential of these extraction methods to produce nutritionally valuable sunflower protein ingredients.

Chapter 2: Literature Review

2.1. Sunflower Seeds and Oil Processing

Sunflower (*Helianthus annuus* L.) is one of the major oilseed crops produced and consumed worldwide, and it is valued primarily for its high oil content and favorable fatty acid composition (Kaur & Ghoshal, 2022; Pilorgé, 2020). Unprocessed sunflower seeds are composed of 10–20% protein, 34–55% fat, 18–26% carbohydrates, 1.1–4.5% chlorogenic acid (CGA), and 2–4% total ash (Kaur & Ghoshal, 2022; Ren et al., 2015). The composition of sunflower seeds can vary depending on the cultivar, growing conditions, dehulling process, and analytical method used.

Industrial sunflower oil production generally involves mechanical, thermal, and chemical operations designed to separate the oil-rich fraction from the other solid seed material. Before oil removal, seeds are typically cleaned, dried, dehulled, cracked, conditioned, and flaked to improve oil release (Le Clef & Kemper, 2015). The dehulling process reduces the fibre-rich hull fraction, while cracking and flaking rupture seed tissues and improve the diffusion of oil out of the solid seed material during mechanical pressing or solvent extraction. Thermal conditioning or cooking is commonly applied before pressing because heat reduces oil viscosity, increases oil droplet size through coalescence, softens the seed flakes, and improves oil flow. However, excessive heat can affect the quality of the remaining SFM and denature its protein.

The first major method for sunflower oil extraction is mechanical pressing, in which prepared sunflower kernels are compressed using expellers or screw presses. In this process, pressure, shear, and friction rupture oil-rich cells and force oil out of the seed matrix, leaving behind a press meal that still contains residual oil and protein (Le Clef & Kemper, 2015). Mechanical pressing can be performed under cold-pressing or hot-pressing conditions. Cold

pressing uses lower temperatures and is often associated with better preservation of heat-sensitive compounds, such as protein, but it usually gives lower oil recovery (Nde & Foncha, 2020). Hot pressing improves oil yield because heat enhances oil flow and cell disruption, but the higher temperature can negatively affect meal proteins by promoting denaturation, aggregation, and lower solubility (Carré, 2021). The second major oil extraction method is solvent extraction, most commonly using hexane. In industrial oil processing, solvent extraction is often applied after mechanical pre-pressing, to reach maximum oil recovery (Le Clef & Kemper, 2015). During solvent extraction, the pre-pressed meal is in contact with hexane in a continuous counter-current extraction system, which dissolves the nonpolar triglycerides and forms an oil-solvent mixture known as the miscella (Carré et al., 2024). The miscella is then separated from the solid meal, and the solvent is removed from both the crude oil and the meal by evaporation and distillation, thereby recovering and reusing hexane (Mosenthin et al., 2016). Hexane extraction is widely used because it is highly efficient, economical, and capable of leaving very low residual oil in the meal; however, hexane is flammable, petroleum-derived, requires strict solvent recovery and safety controls, and denatures the meal protein which reduces further applications, thus this has encouraged research into alternative green solvents and extraction technologies (Cravotto et al., 2022; Mosenthin et al., 2016).

Following oil extraction, the remaining SFM is an important protein-rich by-product. The SFM by-product has an elevated protein content ranging from 30–50% (Alexandrino et al., 2017; Hadidi et al., 2024; Lomascolo et al., 2012). The method used for oil extraction strongly affects the composition, protein content, nutritional value, and techno-functional quality of the resulting SFM. Mechanical, thermal, and chemical processing can alter protein structure and techno-functionality through denaturation, aggregation, oxidation, and changes in protein–phenolic

interactions (Carré, 2021; X. Huang et al., 2024). Currently, SFM is exclusively used as animal feed, despite its potential for higher-value food applications (González-Pérez et al., 2002; Lomascolo et al., 2012). However, the increasing demand for sustainable plant proteins has renewed interest in upgrading sunflower meal into food-grade protein concentrates and isolates, provided that challenges associated with isolating protein from SFM are addressed.

2.2. Sunflower Meal Protein as a Sustainable Source

Protein is a key dietary component and a fundamental building block in meeting the global population's increasing demand for nutritious foods. Furthermore, protein plays an essential role in the human diet as the body depends on it to preserve muscle tissue, support immune function, regulate cellular communication, and restore damaged cells (Carbone & Pasiakos, 2019; P. Li et al., 2007; Wu, 2016). These factors are concomitant with the rising demand for protein from both conventional and alternative sources.

Plant proteins are gaining attention as more sustainable alternatives for animal proteins, offering high nutritional value and techno-functional properties for food applications (Chandran et al., 2023). Among plant protein sources, SFM offers a great advantage because it is protein-rich by-product of oil extraction that can be valorized into a food ingredient with greater economic and nutritional value, while simultaneously improving the sustainability of the oil processing industry (De Oliveira Filho & Egea, 2021; Nevara et al., 2023). Sunflower protein is especially attractive because it has a favorable amino acid profile compared with many other plant proteins and low levels of antinutritional compounds (González-Pérez & Vereijken, 2007; X. Huang et al., 2024; Kaur & Ghoshal, 2022). Another advantage of using sunflower protein is its extremely low allergenicity compared with soy, a major allergenic plant protein source (Beaubier et al., 2021). There are rare reports of IgE-mediated allergic reactions associated with sunflower seeds (Achour

et al., 2021; Lavine & Ben-Shoshan, 2015). Thus, SFPI may offer formulation advantages for consumers avoiding common allergens as compared to other commonly used plant protein sources.

2.2.1. Sunflower Meal Composition

Following oil extraction, SFM is composed mainly of protein, lignocellulosic fibre, residual lipids, minerals, carbohydrates, and phenolic compounds (De Oliveira Filho & Egea, 2021; Lomascolo et al., 2012). The composition of SFM depends strongly on the dehulling process, seed variety, oil extraction method, and processing conditions applied during solvent drying or evaporation (Hadidi et al., 2024; Lomascolo et al., 2012). Protein, ranging from 30–50%, is the most valuable component of SFM, and becomes more concentrated in dehulled and highly defatted meals due to the removal of insoluble fibre and oil.

The protein fractions of SFM is dominated by 11S globulins (helianthinin), accounting for approximately 60–80% of the total sunflower seed protein content, followed by 2S albumins which account for approximately 25–30% (Beaubier et al., 2021; González-Pérez & Vereijken, 2007; Kaur & Ghoshal, 2022). The 11S globulin fraction is a salt-soluble storage protein with a relatively high molecular weight and is structurally comparable to legumin-type proteins found in other oilseeds and legumes (González-Pérez & Vereijken, 2007). These globulins are important for the nutritional value of sunflower protein, but their solubility is strongly affected by pH, ionic strength, and oil and protein processing (González-Pérez et al., 2005; González-Pérez & Vereijken, 2007). In contrast, 2S albumins are lower-molecular-weight, water-soluble proteins that are more resistant to pH changes and heat treatment than helianthinin (González-Pérez et al., 2005). Because of their water-soluble nature, 2S albumins significantly contribute to the solubility of SFPI, thereby

improving dispersion and techno-functionality (González-Pérez et al., 2005; Hadidi et al., 2024; Z. Li et al., 2024).

In addition to protein, SFM contains a fibre fraction derived primarily from the seed's hull and cell wall materials. This fraction includes insoluble polysaccharides (such as cellulose and hemicellulose) and lignin (Liu et al., 2021; Lomascolo et al., 2012). The presence of fibre can be nutritionally beneficial in some applications, but it can limit protein concentration in SFM, reduce digestibility, and interfere with protein isolation (Lomascolo et al., 2012; Murru & Calvo, 2020). Consequently, incorporating processes such as dehulling, milling, and air classification have been investigated to reduce fibrous components and enrich the protein fraction of SFM (Murru & Calvo, 2020). Moreover, the remaining lipid fraction, including neutral lipids and phospholipids, can influence the oxidative stability, flavor, storage of SFM, and efficiency of protein isolation (Kaur & Ghoshal, 2022; Petraru et al., 2021). SFM contains varying amounts of minerals such as phosphorus, potassium, magnesium, calcium, manganese, and zinc (Petraru et al., 2021). Carbohydrates are also present in SFM as structural cell wall polysaccharides such as cellulose, xylans, and xyloglucans, along with soluble sugars and low levels of starch (Bautista et al., 1990; Düsterhöft et al., 1991).

2.2.2. Sunflower Protein Nutritional Quality

2.2.2.1. Amino Acid Composition

The amino acid composition is an important determinant of the nutritional quality of sunflower protein, due to their role in physiological functions such as protein synthesis, tissue maintenance, enzyme and hormone production, and immune regulation (Carbone & Pasiakos, 2019; Wu, 2016). Of the 20 amino acids required by the human body, nine are considered essential: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine.

These amino acids cannot be synthesized by the body in adequate amounts and must be supplied through the diet (Church et al., 2020). A high-quality or complete protein is expected to provide all nine essential amino acids in sufficient amounts to meet requirements, although digestibility and bioavailability must also be considered when comparing protein sources (Chandran et al., 2023; Church et al., 2020).

SFPI has a favourable amino acid profile comparable to other plant protein sources. Various studies reported SFPI contains substantial amounts of essential amino acids, although lysine is consistently identified as the first limiting amino acid (Beaubier et al., 2021; González-Pérez & Vereijken, 2007; Ivanova et al., 2013; Zaky et al., 2022). Among all essential amino acids, SFPI is abundant in leucine, followed by phenylalanine and threonine (Zaky et al., 2022). This pattern suggests that SFPI can contribute meaningfully to food formulations to meet the dietary essential amino acid intake. Furthermore, SFPI is distinguished by its relatively high content of sulfur-containing amino acids, methionine and cysteine, compared with several other plant protein sources such as soy, legumes, and pulses (Beaubier et al., 2021; González-Pérez & Vereijken, 2007; Grela et al., 2017). Therefore, SFPI's methionine and cysteine contents may provide a useful complementary advantage in plant-based food formulations. The non-essential amino acid fraction of SFPI has a high proportion of glutamic acid, followed by aspartic acid and arginine (Z. Li et al., 2024; Zaky et al., 2022). These amino acids are relevant to nutrition and techno-functionality due to their influence on protein charge, which enhances hydration, solubility, and interactions with other food components. The relative proportions of essential and non-essential amino acids may vary among sunflower varieties and processing conditions, with essential amino acids representing 31–34% of the total amount (Z. Li et al., 2024).

Although sunflower meal protein is nutritionally promising, the essential amino acid lysine is recognized as the primary limiting amino acid. This suggests that SFPI may be best used in combination with other plant protein sources that have limited sulfur amino acids, rather than as the sole dietary protein. Blending complementary plant proteins can improve the overall amino acid balance, nutritional quality, and digestibility (Beaubier et al., 2021; Dimina et al., 2022; González-Pérez & Vereijken, 2007). For example, SFPI, which is relatively rich in sulfur-containing amino acids but low in lysine, can be combined with legume proteins, which can provide more lysine but have lower sulfur amino acids.

2.2.2.2. Protein Digestibility

Protein digestibility is an important component of protein quality as it reflects the extent to which dietary proteins are hydrolyzed into absorbable peptides and amino acids during gastrointestinal digestion. For SFM and SFPI, analyzing digestibility is particularly vital as phenolic compounds, residual lipids, and other non-protein components such as fibre can hinder enzyme accessibility and protein hydrolysis (Alexandrino et al., 2017; Bisinotto et al., 2023). In vitro protein digestibility (IVPD) assays are commonly conducted to estimate the susceptibility of food proteins to digestive enzymes (Brodkorb et al., 2019; Minekus et al., 2014). Traditional enzymatic methods include the pepsin-pancreatin digestibility assay, in which protein samples are first exposed to acidic gastric conditions with pepsin and then to intestinal conditions with pancreatin (Akeson & Stahmann, 1964). Another widely used assay is the multienzyme pH-drop method, which uses enzymes such as trypsin, chymotrypsin, and peptidase to estimate digestibility based on the decrease in pH during protein hydrolysis (Hsu et al., 1977; Pedersen & Eggum, 1981). More recently, the standardized INFOGEST static digestion model has been applied to simulate

oral, gastric, and intestinal digestion under more physiologically relevant conditions (Brodkorb et al., 2019).

The digestibility of SFM and SFPI ingredients depends on their processing conditions and protein concentration. In contrast to SFM, SFPI usually has higher digestibility due to protein extraction reducing the proportion of insoluble fibre and other matrix components surrounding the protein (Alexandrino et al., 2017). Various studies reported a high IVPD value for sunflower protein isolates or concentrates produced from defatted SFM (Alexandrino et al., 2017; Öztürk et al., 2024). Further processing of SFPI under mild heat treatments and high-moisture extrusion can improve enzymatic breakdown and IVPD, thereby expanding the use of SFPI in food formulations. Although SFPI often shows high IVPD, protein quality also depends on whether the digested protein supplies indispensable amino acids in adequate proportions. Therefore, the protein digestibility-corrected amino acid score (PDCAAS) is a protein quality index that combines the amino acid score of the first limiting essential amino acid with IVPD, known as the IV-PDCAAS, with values expressed on a scale from 0 to 1, where 1 represents an ideal protein that meets or exceeds the reference amino acid requirement (Marinangeli & House, 2017; Salter & Lopez-Viso, 2021; Schaafsma, 2000). Consequently, the lysine limitation in SFPI results in a lower calculated IV-PDCAAS (Alexandrino et al., 2017; Tessier et al., 2020). Nevertheless, comparing the PDCAAS values for plant and animal proteins as a measure of protein quality illustrates that many traditional plant proteins are less digestible and lack some essential amino acids, thus driving the need for newer plant protein sources and combinations for food formulations (Ismail et al., 2020).

2.2.3. Anti-Nutritional Content

2.2.3.1. Phenolic Compounds

Phenolic compounds present in SFM includes caffeic acids, caffeoylquinic caffeic acid derivates, quinic acid, ferulic acid, and p-coumaric acid (González-Pérez & Vereijken, 2007; Karamać et al., 2012; Wildermuth et al., 2016). In addition, CGA, an ester of caffeic and quinic acids, is the predominant polyphenol associated with sunflower seeds and SFM, accounting for 60–70% of the total phenolic content (Alexandrino et al., 2017; Taha et al., 2011; Wildermuth et al., 2016). From a nutritional perspective, CGA has been widely studied as a bioactive polyphenol with antioxidant and anti-inflammatory properties, where it can reduce oxidative stress and modulate inflammatory pathways, and contributes to reductions in blood pressure (J. Huang et al., 2023; Nguyen et al., 2024; Tajik et al., 2017).

Despite these potential health benefits, CGA presents a major technological and nutritional challenge during the production of SFPI. The relatively high levels of phenolic compounds remaining in SFM after oil extraction can become incorporated into SFPI during aqueous protein extraction methods (Kaur & Ghoshal, 2022; Shchekoldina & Aider, 2014; Wildermuth et al., 2016). SFM has been described as having one of the highest CGA concentrations among oilseed meals, with reported CGA levels around 2–4% (Alexandrino et al., 2017; Shchekoldina & Aider, 2014; Wildermuth et al., 2016). CGA is particularly problematic under alkaline conditions, which is commonly used as a conventional protein extraction method. During alkaline-based extraction, CGA can oxidize to reactive quinones, which interacts with protein groups including the amino groups of lysine and the sulfhydryl group of cysteine, leading to the formation of undesirable green or brown pigments and phenol-protein complexes (Wildermuth et al., 2016; Malik & Saini, 2017; Verde et al., 2022). (Malik & Saini, 2017; Wildermuth et al., 2016). These reactions are responsible

for the characteristic dark green discolouration observed in SFPI ingredients, which reduces the sensory quality and consumer acceptability. In addition, phenol-protein interactions may reduce available lysine content, which is limiting in SFPI, and alter protein solubility, digestibility, and techno-functional properties (González-Pérez & Vereijken, 2007; X. Huang et al., 2024; Malik & Saini, 2017). The interaction between CGA and sunflower proteins can occur through both non-covalent and covalent mechanisms, depending on pH and oxidation conditions. CGA can bind to SFPI at neutral pH via non-covalent interactions, such as hydrogen bonding, resulting in larger phenolic-protein complexes (Karefyllakis et al., 2017). Under alkaline conditions, CGA forms covalent bonds with proteins, where higher amounts of phenol-protein complexes can promote green discolouration and reduce techno-functional properties (Jia et al., 2022; Wildermuth et al., 2016). In light of this, food-grade valorization of SFM often requires pre-processing such as dehulling, dephenolization, controlled extraction conditions, or other purification methods to improve colour, digestibility, and techno-functional performance (González-Pérez & Vereijken, 2007; Hadidi et al., 2024; X. Huang et al., 2024; Kaur & Ghoshal, 2022). Several studies have investigated methods to reduce CGA content in SFPI, with improved protein content and techno-functionality observed (Alexandrino et al., 2017; Malik & Saini, 2017).

Several dephenolization methods have been investigated to reduce CGA in SFM before or during SFPI production. These methods include pre-extraction with aqueous organic solvents such as ethanol or methanol, the use of salts such as sodium bisulfite, reducing agents to limit oxidative reactions, membrane filtration, controlled pH extraction, enzymatic hydrolysis of CGA, and post-isolation washing with solvents such as isopropanol (Alexandrino et al., 2017; González-Pérez et al., 2002; X. Huang et al., 2024; Saricaoglu et al., 2023; Wildermuth et al., 2016). However, phenolic removal may also reduce antioxidant capacity and alter techno-functionality; thus, the

dephenolization process must have a balance between improving protein quality and retaining bioactive compounds (González-Pérez & Vereijken, 2007; Saricaoglu et al., 2023). Moreover, no standardized dephenolization method has yet been established, and its effect on the resulting SFPI can vary.

2.2.3.2. Trypsin Inhibitory Activity

Compared with phenolic compounds, trypsin inhibitory activity (TIA) in SFM and SFPI has received less attention; however, due to the presence of sunflower-derived trypsin inhibitors such as SFTI-1, TIA should be evaluated when developing food-grade sunflower protein ingredients. Trypsin inhibitors are anti-nutritional factors that can reduce protein hydrolysis by inhibiting digestive proteases, particularly trypsin and chymotrypsin (De Oliveira Filho & Egea, 2021). The presence of TIA can lower protein digestibility by reducing the action of intestinal proteases, amino acid availability, and negatively affect overall protein quality (Avilés-Gaxiola et al., 2018; Sarwar Gilani et al., 2012). Trypsin inhibitor activity is usually reported as trypsin inhibitor units (TUI) per unit of sample, although reported values can vary depending on assay conditions, calculation method, and the way inhibitory activity is expressed (Liu et al., 2021).

SFM is considered to have fewer antinutritional factors, such as TIA, than other plant-based protein sources. Some literature reviews describe SFM as relatively low in, or nearly free from, trypsin inhibitors (Wildermuth et al., 2016). However, a study investigating antinutrients in various sunflower seed varieties identified one major and two minor groups of trypsin inhibitors which are associated with water-soluble seed protein fractions (Konarev et al., 2000). The major trypsin inhibitor in SFM is sunflower trypsin inhibitor-1 (SFTI-1), a small 14-amino-acid bicyclic peptide originally isolated from sunflower seeds with strong TIA (Luckett et al., 1999). SFTI-1 is stabilized by a head-to-tail cyclic backbone and a disulfide bond, which are structural features that

contribute to its rigidity and high inhibitory potency (Colgrave et al., 2010; Korsinczky et al., 2001). Because of its small size and stability, SFTI-1 has been studied as a peptide scaffold in biochemical and pharmaceutical research; however, its nutritional significance in SFM and SFPI remains less studied (Colgrave et al., 2010; Luckett et al., 1999).

Trypsin inhibitors may remain in SFM after oil extraction because they are non-lipid-soluble compounds. During SFPI production, these inhibitors may be co-extracted with the soluble protein fraction, particularly during aqueous or alkaline extraction (Konarev et al., 2000). Therefore, residual TIA should still be assessed in SFPI, especially when mild or low-temperature extraction methods are used ((De Oliveira Filho & Egea, 2021; Wildermuth et al., 2016). Testing TIA in SFPI is particularly relevant as it is limited by lysine, so any reduction in digestibility could further reduce protein quality measurements such as IV-PDCAAS. Furthermore, protein processing conditions can influence both TIA and protein functionality. Heat treatment and extrusion may reduce TIA and improve digestibility, but excessive heating can denature proteins and reduce techno-functional properties (Avilés-Gaxiola et al., 2018; Öztürk et al., 2024). Therefore, SFPI production requires a balance between reducing anti-nutritional activity and preserving the techno-functional properties needed for food applications.

2.2.4. Structural Characterization

The secondary and tertiary structures of SFPI are important determinants of its nutritional quality and techno-functional performance. The Protein secondary structure refers to conformations of the polypeptide backbone, mainly α-helices, β-sheets, β-turns, and random coils, whereas tertiary structure refers to the three-dimensional arrangement of the protein, including the spatial environment of aromatic amino acids, hydrophobic residues, and disulfide bonds (Kelly et al., 2005; Lan et al., 2024). Fourier-transform infrared spectroscopy (FTIR) is commonly used to

estimate secondary structural components in the amide I region between 1600–1700 cm^{-1}, as this region is mainly associated with C=O stretching vibrations of the peptide backbone, which are indicative of protein secondary structures (Lan et al., 2024). In addition, circular dichroism (CD) spectroscopy is also widely used for protein structural characterization, with far-UV CD providing information on secondary structure and near-UV CD providing a fingerprint of tertiary structure through the local environments of phenylalanine, tyrosine, tryptophan, and disulfide bonds (Kelly et al., 2005).

The structure of SFPI is strongly influenced by its major protein fractions, particularly 11S globulins (helianthinin) and 2S albumins. Helianthinin is the predominant sunflower storage protein and has a quaternary structure that can shift depending on pH, ionic strength, and temperature (González-Pérez et al., 2004; González-Pérez & Vereijken, 2007). SFPI is characterized by a secondary structure high in β-sheet content, followed α-helices, and variations in sulfhydryl and disulfide bond contents depending on sunflower varieties (Z. Li et al., 2024). These structural features are important to assess because β-sheet-rich and disulfide-stabilized proteins are often more compact and less soluble, and partial unfolding can expose hydrophilic or hydrophobic groups that alter solubility, interfacial adsorption, emulsification, foaming, and gelation behavior and techno-functionality (Beaubier et al., 2021; González-Pérez et al., 2004; Z. Li et al., 2024).

The extraction method is one of the main factors affecting the secondary and tertiary structures of SFPI. Conventional alkaline-based extraction followed by isoelectric precipitation is widely used in the industry; however, exposure to high pH and subsequent precipitation near the isoelectric point can promote unfolding, hydrophobic interactions, and protein aggregation (González-Pérez et al., 2004; Ivanova et al., 2012; Yang et al., 2024). These structural changes

can reduce solubility and techno-functional properties with excessive (Beaubier et al., 2021; Yang et al., 2024). In contrast, milder extraction approaches can better preserve native protein conformations by avoiding severe pH conditions (X. Huang et al., 2024; Pickardt et al., 2009). In the context of SFPI, CGA can also influence structure by binding to proteins through non-covalent or covalent interactions under alkaline conditions (Jia et al., 2022; Karefyllakis et al., 2017). These phenolic-protein interactions can alter secondary and tertiary structure by changing protein folding, surface charge, hydrophobicity, and aggregation behavior (Jia et al., 2022; Malik & Saini, 2017). Therefore, emerging extraction and modification methods are increasingly being studied to improve SFPI structure while preserving techno-functionality.

2.2.5. Techno-Functional Properties

The techno-functional and sensory properties of SFPI are based on various intrinsic factors such as size, shape, structure, amino acid composition, charge distribution, and extrinsic extraction factors such as pH, temperature, and chemical reagents used (Ivanova et al., 2012; Kaur & Ghoshal, 2022).

2.2.5.1. Solubility

Protein solubility is one of the most important functional properties of SFPI because it strongly influences their performance in food systems, particularly in emulsions and foams. High solubility enables protein molecules to disperse uniformly in water and enhances their ability to migrate and adsorb at oil-water or air-water interfaces, which is vital for emulsion and foam formation (Beaubier et al., 2021). In SFPI, solubility is closely related to the balance between protein-water and protein-protein interactions, which are affected by pH, ionic strength, protein structure, and the presence of non-protein compounds such as phenolics (González-Pérez & Vereijken, 2007; Kaur & Ghoshal, 2022). Sunflower proteins exhibit lower solubility near their

isoelectric region at pH 4–6, because the protein gains a neutral charge and electrostatic repulsion is minimized, thereby promoting aggregation and precipitation (Beaubier et al., 2021; Kaur & Ghoshal, 2022). In contrast, solubility increases under alkaline conditions because proteins carry a stronger net negative charge, which increases electrostatic repulsion and protein hydration. Low solubility at neutral pH can pose challenges, as many food products are formulated near this pH level, making solubility improvement a key requirement for expanding SFPI applications in food.

Several factors can hinder SFPI solubility. The predominance of 11S globulins, which are less soluble near their isoelectric points, contributes to protein aggregation and reduced SFPI solubility in acidic and neutral pH values. Furthermore, processes such as heat treatment, alkaline extraction, and isoelectric precipitation can promote denaturation, hydrophobic interactions, and formation of insoluble aggregates (Beaubier et al., 2021; González-Pérez & Vereijken, 2007; X. Huang et al., 2024). Therefore, the method used to produce SFPI is important to consider. Moreover, high concentrations of CGA can also modify solubility through phenolic-protein interactions. To overcome solubility limitations, controlled enzymatic hydrolysis, ultrasound treatment, heat treatment, and extraction technologies to replace alkaline-based extraction can be investigated (Beaubier et al., 2021; González-Pérez & Vereijken, 2007; X. Huang et al., 2024).

2.2.5.2. Water and Oil Holding Capacity

Water-holding capacity (WHC) and oil-holding capacity (OHC) are important functional properties for utilizing SFPI as a food ingredient. These properties can influence hydration, texture, mouthfeel, juiciness, fat retention, and overall performance in various food matrices that require water or oil retention (X. Huang et al., 2024; Z. Li et al., 2024; Muhammad et al., 2026). WHC refers to a protein ingredient's ability to absorb and retain water, whereas OHC describes its ability to absorb and retain oil. These properties are influenced by protein composition, degree of

denaturation, surface hydrophobicity, hydrophilic and hydrophobic amino acid composition, particle size and porosity, pH, and protein extraction method (X. Huang et al., 2024; Ivanova et al., 2014; Z. Li et al., 2024).

WHC depends on the ability of hydrophilic amino acids, charged groups, and exposed peptide bonds to interact with and retain water. Proteins with more polar groups can bind water through hydrogen bonding and electrostatic interactions, while particle size, porosity, and protein network structures contribute to water retention through physical entrapment within the protein matrix (Foegeding & Davis, 2011; Y. Zhang et al., 2021). In SFPI, differences in extraction method and subsequent phenolic content have been shown to affect hydration-related properties, including water absorption and WHC (Ivanova et al., 2014; Salgado et al., 2011). Furthermore, WHC can be reduced for SFPI if it has low solubility, and if the extraction method forms compact protein aggregates. On the other hand, OHC reflects SFPI's ability to absorb and retain oil through hydrophobic interactions, physical entrapment, and retention within the protein particles. Proteins with exposed hydrophobic amino acid residues generally show stronger OHC, as the nonpolar side chains can interact with lipid molecules. Therefore, moderate unfolding during extraction or processing may improve OHC by exposing hydrophobic regions, while excessive aggregation can reduce oil binding by limiting accessible surface area.

Compared with other plant protein isolates, SFPI exhibits high WHC and OHC, although reported values vary widely depending on extraction and processing conditions. Studies comparing SFPI to soybean protein isolates found that it has comparable WHC and a higher OHC (Khalil et al., 1985). Overall, SFPI has promising WHC and OHC for use in high-moisture and fat-containing food systems, such as meat analogs, but these properties depend strongly on extraction method,

residual phenolics, protein structure, pre- or post-extraction treatments, and the degree of extraction-induced unfolding or aggregation.

2.2.5.3. Foaming Capacity and Stability

Foaming capacity and stability are vital for protein ingredients used in aerated food systems, including whipped toppings, desserts, bakery products, beverages, and other foam-based formulations. Foaming capacity refers to the ability of proteins to incorporate air during whipping, mixing, or homogenization, whereas foaming stability refers to the ability of the formed foam to resist collapse and bubble coalescence over time (Damodaran, 2005; Muhammad et al., 2026). Proteins contribute to foam formation by diffusing and adsorbing at the air-water interface, reducing surface tension, unfolding or rearranging at the interface, and forming a cohesive viscoelastic film around air bubbles (Damodaran, 2005; Rodriguezpatino et al., 2007; Subaşı et al., 2020). Therefore, foaming capacity and stability are strongly affected by protein solubility, structure, surface hydrophobicity, hydrophilic and hydrophobic amino acid composition, aggregation state, pH, ionic strength, and the extraction method used (Beaubier et al., 2021; X. Huang et al., 2024; X. Zhang et al., 2025).

Foaming capacity for SFPI depends largely on its solubility at different pH levels and on the degree of unfolding and aggregation that occur during extraction. SFPI are considered promising foaming agents because their 2S albumin and 11S globulin fractions can contribute to interfacial activity; however, their foaming capacity may be limited when solubility is low near the isoelectric pH, or when extraction causes excessive aggregation (Beaubier et al., 2021; González-Pérez & Vereijken, 2007). Beaubier et al. (2021) compared SFPI with commercial pea protein isolate and partially hydrolyzed soy protein and reported that SFPI exhibited good foaming capacity and foam stability, indicating its potential as a functional ingredient for aerated food

systems. Similarly, earlier studies on SFPI reported that partial unfolding can improve protein flexibility and enhance adsorption at the air-water interface, which increases foaming performance (González-Pérez & Vereijken, 2007). Foaming stability is the ability of the adsorbed protein layer to maintain bubble structure after foam formation. A stable foam requires proteins to form a strong, cohesive, and viscoelastic interfacial film that resists collapse, gas diffusion, and bubble coalescence (Damodaran, 2005; Rodriguezpatino et al., 2007). SFPI can provide improved foaming stability and form strong networks around foam bubbles compared to other plant sources; however, it can be reduced by low protein solubility at the isoelectric point and excessive aggregation during extraction (González-Pérez & Vereijken, 2007; Kaur & Ghoshal, 2022). Furthermore, phenolic compounds can influence foaming capacity and stability, due to CGA interacting with proteins and modifying solubility, aggregation, and surface properties (Jia et al., 2022; Karefyllakis et al., 2017).

2.2.5.4. Emulsification Activity and Stability Index

The emulsification activity index (EAI) and emulsification stability index (ESI) are important indicators of a protein's ability to form and stabilize oil-in-water emulsions. EAI is the ability of proteins to rapidly adsorb at the oil-water interface and generate interfacial area during homogenization, whereas ESI is the resistance of the formed emulsion to destabilization and separation over time. Protein-based emulsification depends on several physicochemical factors, including protein solubility, surface charge, molecular flexibility, hydrophobic and hydrophilic amino acid composition, protein conformation and structure, and the ability of proteins to form a protective interfacial layer around oil droplets (Damodaran, 2005; X. Huang et al., 2024). In general, proteins with good solubility and balanced amphiphilic characteristics can readily diffuse

to the oil-water interface, unfold, reduce interfacial tension, and form a film that enhances emulsification (Damodaran, 2005; Karefyllakis et al., 2017).

The EAI for SFPI is closely associated with the structural and solubility behavior of the major sunflower protein fractions, 11S globulins (helianthinin) and 2S albumins. González-Pérez et al. (2005) reported that SFPI can form stable emulsions, although the emulsifying behavior depends on pH and the composition of the protein fraction. The 11S globulin fraction can contribute to interfacial film formation, while its low solubility near the isoelectric region can reduce the amount of protein available to adsorb at the oil-water interface (González-Pérez et al., 2005; González-Pérez & Vereijken, 2007). To balance this, the 2S albumin fraction can support the EAI by increasing the diffusion and adsorption at the oil-water interface due to its high solubility at low pH levels (González-Pérez et al., 2005; Ivanova et al., 2013). On the other hand, ESI in SFPI depends on the strength and integrity of the adsorbed protein layer around oil droplets. A stable emulsion requires the interfacial film to resist droplet coalescence and phase separation during storage. Factors that can hinder ESI include low protein solubility, excessive aggregation, poor molecular flexibility, insufficient surface charge, and a high concentration of residual non-protein components such as phenolic compounds that interfere with protein adsorption or film formation (González-Pérez & Vereijken, 2007; X. Huang et al., 2024; Malik & Saini, 2017). SFPI has a high ESI due to its ability to stabilize droplets once adsorbed at the oil-water interface (Beaubier et al., 2021). Removal of polyphenols from sunflower seed and meals can improve emulsifying activity and emulsion stability of the resulting protein isolates (Malik & Saini, 2017). However, low phenolic interactions that form non-covalent complexes between SFPI and CGA can decrease interfacial tension and produce oil-in-water emulsions with high stability, suggesting that the effect of phenolics depends on concentration and binding mechanism (Karefyllakis et al.,

2017). Furthermore, since SFPI generally has a lower emulsifying capacity but excellent emulsion stability, they can be combined with other complementing plant protein sources in food formulations.

2.2.5.5. Least Gelation Concentration

Protein gelation is an important functional property for formulating structured foods with certain textural characteristics. Proteins are utilized to form cohesive networks that contribute to firmness, elasticity, water retention, and overall texture in food systems. Gelation is commonly assessed using the least gelation concentration (LGC), which is the lowest protein concentration required to form a self-supporting gel that does not flow or break when handled (Jia et al., 2022; K. K. Ma et al., 2022). A lower LGC indicates a stronger gel-forming ability as less protein is needed to form a stable three-dimensional network. Protein gelation generally involves heat-induced unfolding, exposure of reactive groups, aggregation, and formation of a three-dimensional network through hydrophobic interactions, hydrogen bonding, electrostatic interactions, and disulfide linkages (Khalesi et al., 2024; Y. Ma & Chen, 2023; Totosaus et al., 2002). During heating, globular proteins partially unfold, exposing buried hydrophobic regions and sulfhydryl groups that promote protein-protein interactions and network formation. The final gel structure depends on controlled unfolding that promotes network formation, whereas excessive aggregation can produce coarse, weak, or brittle gels with poor water retention (Khalesi et al., 2024; Totosaus et al., 2002).

SFPI gelation is influenced by protein concentration, pH, protein extraction method, protein purity, and residual non-protein components such as phenolic compounds (Malik & Saini, 2017; Salgado et al., 2012). Polyphenols reduced the storage and loss modulus and produced more viscous, weak gels, suggesting that phenolic compounds can severely interfere with protein-protein

interactions required for strong gel network formation (Malik & Saini, 2017). CGA, the dominant phenolic compound in SFPI, can have both beneficial and detrimental effects on gelation depending on concentration and binding mechanism. Higher chlorogenic acid-to-protein ratios reduced gel strength and caused more pronounced discolouration, which is undesirable in food formulations (Jia et al., 2022). Compared with other plant protein isolates, SFPI shows promising but variable gelation behavior. Some studies found that SFPI gelation was lower than that of soy protein isolate, while other studies report that SFPI can form gels at comparable concentrations when protein purity is high and phenolic content is controlled (Kaur & Ghoshal, 2022; Malik & Saini, 2017).

2.2.6. Economic Relevance

SFM has a strong economic relevance because it is produced in large quantities as a by-product of the oil extraction industry, but it is still used mainly as a lower-value animal feed ingredient. Globally, approximately 20 million tonnes of SFM were produced in 2019–2020 and accounted for 5.6% of the total oilseed meal production, ranking after soybean and rapeseed meals (Pilorgé, 2020). More recent USDA estimates report global SFM production of 21.41 million metric tonnes in 2024–2025, with production forecast to increase to 22.24 million metric tonnes in 2025–2026, and 24.76 million metric tonnes in 2026–2027 (USDA-FAS, 2026). In North America, sunflower production is smaller than that of soybean and canola, but it remains economically important in specific regions. In the United States, sunflower production reached 2.32 billion pounds in 2025, with a crop value of 516.6 million USD, while Canadian sunflower seed production was estimated at 60 thousand tonnes in 2025, with 90% of production concentrated in Manitoba (Manitoba Agriculture, 2025; Statistics Canada, 2026; USDA-FAS, 2026).

The current use of SFM as animal feed limits its economic value, as it is generally priced at a discount to soybean meal due to its lower lysine content, higher fibre content, and less consistent supply. In the United States, SFM was priced at approximately 163 USD per metric tonne in the 2024–2025 marketing year, showing its relatively low value as a bulk feed ingredient (USDA-Economic Research Service, 2026). However, SFM contains approximately 30–50% protein depending on dehulling and oil extraction conditions, making it a promising raw material for producing higher-value SFPI (De Oliveira Filho & Egea, 2021; Hadidi et al., 2024). Upgrading SFM into food-grade SFPI aligns with the sustainable circular economy and zero-waste processing approaches by converting an underutilized oilseed by-product into a functional plant protein ingredient (Grahovac et al., 2025). Nevertheless, the economic feasibility of SFPI production depends on achieving high protein recovery, reduced phenolic content, good digestibility, and competitive techno-functional properties while controlling extraction energy and wastewater-management costs (González-Pérez & Vereijken, 2007; Hadidi et al., 2024; Murru & Calvo, 2020).

2.2.7. Applications of Sunflower Protein as a Food Ingredient

The techno-functional properties of SFPI have potential in food applications such as bakery products, meat analogs, beverages, emulsified sauces, and structured protein foods (De Oliveira Filho & Egea, 2021; Gültekin Subaşı et al., 2022). In current literature, sunflower proteins have most commonly been investigated as ingredients for protein enrichment and techno-functional improvement rather than as widely commercialized food proteins. Some examples of SFPI application in food products include a study that investigated its techno-functionality for wheat bread production, illustrating that SFPI can be incorporated into cereal-based foods to improve nutritional value when phenolic compounds are reduced (Shchekoldina & Aider, 2014). Similarly,

Mohammed et al. (2018) reported that partial substitution of wheat flour with SFPI increased bread protein and amino acid content, although higher inclusion levels affected bread quality. More recently, SFM ingredients have been investigated for meat analog applications, including high-moisture extrusion systems, where they can contribute to fibrous structure formation (Singh et al., 2024).

2.2.8. Sunflower Meal Protein Production Challenges in Current Literature

It is important to note some of the limitations of using SFM as a protein source. Firstly, conventional sunflower oil extraction often uses high-temperature conditioning (80–90 °C), hot pressing (90 °C), and toasting steps (100–110 °C). During these operations, the heat, pressure, and shear can promote protein denaturation, unfolding, and aggregation; resulting in protein fractions with reduced techno-functionality (Carré, 2021; Carré et al., 2024; X. Huang et al., 2024). The desolventizing and toasting steps to remove residual hexane can particularly affect protein structure and reduce the protein quality in the final SFM (Le Clef & Kemper, 2015; Mosenthin et al., 2016).

Secondly, SFM contains a high level of phenolic compounds, particularly CGA, which is a major barrier to producing food-grade SFPI. During alkaline protein extraction, CGA is susceptible to oxidation, which generates reactive quinones that can bind to protein side chains. These reactions promote the formation of phenolic-protein complexes, often resulting in dark green discolouration and deterioration of the protein isolate's functional performance and overall quality (Malik & Saini, 2017; Wildermuth et al., 2016).

2.3. Plant Protein Extraction and Processing

2.3.1. Conventional Alkaline-Based Extraction

This conventional method is typically comprised of alkaline-based extraction followed by isoelectric precipitation, and it is widely used for producing plant-based protein ingredients in the industry because it is simple, inexpensive, scalable, and compatible for isolating proteins from various plant sources (Chandran et al., 2023). The method is based on protein solubilization under alkaline conditions (pH 9), where proteins carry a strong net negative charge, have increased electrostatic repulsion, and become more soluble and extractable (Chandran et al., 2023; Yao et al., 2023). This is followed by lowering the pH to the isoelectric point (pH 4–5), where proteins are electrically neutral, causing them to aggregate and precipitate. In a typical industrial process, the defatted plant meal is dispersed in water with alkaline NaOH solution, then extracted under controlled pH, time, and temperature conditions. The dispersion is then centrifuged to remove insoluble material, acidified to precipitate the proteins, washed, and dried to obtain a protein concentrate or isolate. Therefore, the efficiency of alkaline-based extraction is affected by pH, extraction time, temperature, and the composition of the raw plant material. Various studies have investigated different alkaline extraction conditions to determine their effects on protein yield and techno-functional properties. It has been found that increasing the extraction pH improves protein solubilization and recovery; however, this improvement is accompanied by reduced techno-functionality due to higher alkaline conditions promoting protein unfolding and aggregation.

The isoelectric precipitation approach is particularly effective for recovering globulin-rich protein fractions. This is because many plant-based globulins, including those in oilseeds, acquire a neutral charge and precipitate at pH 4–5, whereas albumins remain soluble in the supernatant. This selective extraction and precipitation of globulins has been reported for various plant sources

such as pea, lentil, and sunflower proteins (Beaubier et al., 2021; X. Huang et al., 2024; Nadeeshani et al., 2026; Schmidt et al., 2022). Alkaline-based extraction is particularly applicable to sunflower proteins, as its major storage fraction, 11S globulin helianthinin, has an isoelectric region around pH 4–6. Ivanova et al. (2012) found that sunflower protein extractability increased with higher pH, with the highest yield of approximately 60% obtained at pH 10 in the presence of 7.5–12.5% NaCl.

Despite the method's apparent efficiency and widespread industrial adoption, alkaline extraction-isoelectric precipitation can adversely affect protein structure and functionality. The method is favored because alkaline pH increases protein ionization and solubility, allowing higher protein recovery at relatively low cost. However, these extraction conditions cause protein unfolding, expose hydrophobic and reactive sulfhydryl groups, and promote aggregation through hydrophobic interactions or intermolecular disulfide bond formation (Yao et al., 2023). As a result, these negative changes can reduce protein techno-functionality, particularly solubility and emulsifying performance, alter protein structure, and lower nutritional quality.

While sunflower proteins are more extractable at high pH levels, the resulting SFPI is dark-coloured, oxidized, and has reduced techno-functionality, thus making it unsuitable for food applications (Alexandrino et al., 2017; Ivanova et al., 2012). Alkaline-based extraction poses a major challenge for producing SFPI, due to the presence of CGA which is abundant in SFM (González-Pérez & Vereijken, 2007; X. Huang et al., 2024; Malik & Saini, 2017). Under alkaline conditions, CGA can oxidize to o-quinones, which react with protein functional groups such as amino, thiol, indole, and imidazole groups, causing dark green or brown discolouration, reduced solubility, decreased digestibility, altered protein structure, and changes in functional properties (X. Huang et al., 2024; Malik & Saini, 2017). Consequently, alkaline extraction is often combined with pre-extraction dephenolization steps such as aqueous ethanol washing, salt-assisted

extraction, membrane filtration, or other phenolic-removal strategies, which add to the burden of utilizing this protein extraction method for sunflower (Alexandrino et al., 2017; Bau et al., 1983; Wildermuth et al., 2016).

2.3.2. Salt-Based Extraction

Salt-based extraction is a wet protein isolation method used to recover plant proteins, particularly salt-soluble globulin fractions. This method generally involves dispersing defatted meal in an aqueous salt solution, adjusting the pH to a mildly acidic or neutral condition, stirring under controlled temperature and time, then separating the insoluble non-protein materials by centrifugation or filtration, and finally drying. Unlike conventional alkaline extraction, salt-based extraction can be performed under milder pH conditions, which may reduce protein denaturation and limit the oxidation of phenolic compounds during sunflower protein isolation (Albe Slabi et al., 2020; Ivanova et al., 2012). The mechanism of salt extraction is based on ionic strength effects, in which salt ions reduce electrostatic interactions among proteins, thereby improving the solubility and release of salt-soluble proteins. At moderate salt concentrations, the salting-in effect can increase protein extractability; however, excessive salt concentration may reduce solubility through the salting-out phenomenon, leading to aggregation through stronger protein-protein interactions (Arakawa & Timasheff, 1984; Y. Zhang et al., 2025).

Monovalent salt extraction with NaCl has been studied more extensively for sunflower proteins than with other salts. This approach is particularly relevant to SFPI production because sunflower meal is rich in 11S globulin helianthinin, a salt-soluble storage protein. (Ivanova et al., 2012) reported that NaCl-based extraction at pH 5–7 improved the isolation of SFPI while reducing phenolic-protein interactions. Various studies have investigated and optimized NaCl salt-based extraction at pH 7.3, combined with different processes, and reported good protein yields

(Albe Slabi et al., 2020; Beaubier et al., 2021). These studies illustrated that NaCl-assisted extraction can recover both globulin and albumin fractions under milder conditions than strong alkaline extraction, making it useful for producing SFPI with improved colour and reduced alkaline damage. On the other hand, divalent salt extraction, such as with $MgCl_2$, is not commonly investigated in the literature, but it is relevant for producing SFPI because helianthinin is salt-soluble and sensitive to ionic strength. This method follows the same principle as monovalent salt extraction; however, Mg^{2+} has a higher charge density than Na^+ and can more strongly reduce electrostatic interactions and increase protein hydration (Arakawa et al., 1990). Therefore, $MgCl_2$ can improve extraction efficiency at lower molar concentrations. However, optimization of the divalent salt-based extraction method, particularly salt concentration, is often necessary, as divalent cations can also promote ion bridging between negatively charged protein groups, leading to aggregation and reduced solubility (Arakawa et al., 1990; Tiong et al., 2026).

The main advantage of salt-based extraction for SFPI is that it can improve protein recovery at milder pH, thereby reducing the dark green or brown discolouration associated with CGA oxidation under alkaline conditions. It can also help preserve more native-like protein structures and retain both 11S globulins and water-soluble albumins that may otherwise be lost during isoelectric precipitation (Beaubier et al., 2021). However, salt-based extraction also has some limitations. The process may require large volumes of saline solution, and the final SFPI must be desalinated by dialysis or ultrafiltration. This can increase processing cost, energy use, water use, and produce wastewater with a high salt load (Albolafio et al., 2021; Beaubier et al., 2021). In addition, if not removed properly, residual salt can affect protein purity, flavor, and techno-functionality (Beaubier et al., 2021; Karaca et al., 2011; Y. Zhang et al., 2025). Therefore, salt-based extraction is a promising alternative to conventional alkaline extraction for SFPI

production, but its success depends on balancing protein yield, salt removal, functionality, and overall process sustainability.

2.3.3. Deep Eutectic Solvent Extraction

Natural DES extraction has also been investigated as a greener alternative to conventional alkaline extraction. DES are formed by mixing a hydrogen-bond acceptor, commonly choline chloride, with a hydrogen-bond donor such as glycerol, glucose, urea, or organic acids (Hewage et al., 2022; Rente et al., 2021). The strong hydrogen-bonding interactions between the DES components produce a liquid system with a melting point lower than that of the starting compounds, allowing DES to extract proteins under relatively mild thermal conditions (Bowen et al., 2022; Ling & Hadinoto, 2022; Parodi et al., 2023). Choline chloride-glycerol is one of the most common DES systems used for protein extraction because it is relatively inexpensive, has low volatility, is less hazardous than many organic solvents, and contains food-compatible or naturally derived components (Bowen et al., 2022; Grudniewska et al., 2018; Hewage et al., 2022). Prior to protein extraction, the DES system is prepared by heating and stirring the hydrogen-bond acceptor and donor until a homogeneous liquid forms; the water content is then adjusted. For protein extraction, the defatted meal is dispersed in the DES system, where protein is extracted under controlled temperature, time, solid-to-solvent ratio, then separated from insoluble residues by centrifugation, followed by dialysis and drying (Bowen et al., 2022; Grudniewska et al., 2018; Nadeeshani et al., 2026). The extraction mechanism is related to the ability of the DES hydrogen-bonding network to interact with proteins and the surrounding meal matrix. Choline chloride-glycerol DES systems can disrupt protein-matrix interactions, thereby improving protein solubilization (Grudniewska et al., 2018; Wong et al., 2025). In addition, optimizing the water content added to the DES system is important. Low levels of water incorporation can reduce DES

viscosity and improve mass transfer, whereas excessive dilution may weaken the DES system and reduce its extraction performance.

The use of choline chloride–glycerol DES has been investigated for protein extraction from several oilseed materials such as rapeseed, flax, camelina, and sunflower, where enhanced extraction yield and protein content were found in certain optimized conditions (Grudniewska et al., 2018; Parodi et al., 2023). These studies support the use of DES as a green alternative for valorizing oilseed meals into high value protein ingredients. The efficiency of DES protein extraction depends on the protein material, DES composition, DES water content, and processing temperature (Grudniewska et al., 2018; Parodi et al., 2023). For SFPI, DES-based extraction is especially relevant because it can reduce some of the drawbacks associated with strong alkaline extraction. The DES system can extract SFPI with high protein yield and purity under milder conditions, thereby limiting the alkaline-induced protein denaturation and phenolic oxidation while preserving protein structure. Karabulut et al. (2025) reported that DES-extracted SFPI retained more secondary structural features than conventionally alkaline-extracted SFPI, while alkaline extraction caused greater structural disruption, aggregation, and lower digestibility.

Despite these advantages, DES-based extraction still has limitations that must be addressed before it can be widely applied for protein production. Many DES systems have relatively high viscosity, which can slow mass transfer and complicate mixing; thus, appropriate water addition and heating are required to improve DES formulation and protein extraction efficiency (Bowen et al., 2022; Sharma et al., 2023). Protein recovery from DES extracts also requires downstream separation steps, such as dialysis or ultrafiltration, which may increase processing cost and water use. In addition, DES residues in food-grade protein ingredients must be carefully evaluated for safety, sensory impact, and regulatory acceptance, even when the DES components are considered

to be of natural origin (Bowen et al., 2022; Wong et al., 2025). Although DES systems are often described as recyclable extraction solvents, evidence for their recovery and reuse in protein extraction systems remains limited. Therefore, DES extraction is a promising green alternative for producing SFPI from SFM, but its practical use will depend on optimizing DES composition, extraction conditions for high protein recovery and functionality, and sustainability of solvent recycling.

Chapter 3: Valorization of Commercial Sunflower Meal Using Sustainable Extraction Technologies to Enhance Techno-Functional and Physicochemical Properties

Zainab Husain [ab], Nandika Bandara [ab]

[a] Department of Food and Human Nutritional Sciences, Faculty of Agricultural and Food Sciences, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada

[b] Richardson Centre for Food Technology and Research, 196 Innovation Drive, Winnipeg, Manitoba R3T 2N2, Canada

3.1. Abstract

The growing global protein demand pushes the need for sustainable plant-based ingredients as alternatives to conventional sources. Sunflower, a widely cultivated oilseed, produces defatted SFM as a processing by-product. SFM contains approximately 30–40% protein on a dry-weight basis but remains underutilized. This study evaluated three green extraction technologies for producing SFPI from commercial SFM: divalent salt-based extraction (DV-SFPI), monovalent salt-based extraction (MV-SFPI), and DES extraction (DES-SFPI). The performance, techno-functionality, and structural properties of extracted SFPI were compared to the industrial-standard alkaline extracted SFPI (ALK-SFPI) to determine their potential applications. The data were analyzed with ANOVA followed by Tukey's test (n=3), with statistical significance difference at $p < 0.05$. The alternative extraction methods produced isolates with significantly greater protein purity than alkaline extraction. Protein contents of 90.18 ± 0.48%, 90.06 ± 1.01%, and 88.46 ± 1.38% were obtained for MV-SFPI, DV-SFPI, and DES-SFPI, respectively, compared with 69.35 ± 0.49% for ALK-SFPI. Protein recovery and extraction yield were also significantly enhanced,

where DES-SFPI and MV-SFPI achieved the greatest results. Both salt-based extraction methods significantly reduced the residual fat content to approximately 6% compared to 15% in the original SFM. Moreover, the alternative extraction methods had enhanced oil-holding capacity, foaming capacity, and emulsification properties at specific pH levels, while the other techno-functional properties were comparable to ALK-SFPI. DV-SFPI also displayed the lightest off-white appearance, with an L* value of 84.07 ± 0.23. This colour improvement was associated with the exclusion of CGA, a green-pigmented compound retained during alkaline extraction. Overall, these alternative extraction methods demonstrate potential for valorizing SFM into a higher-value protein ingredient with improved protein purity, protein recovery rate, extraction yield, and comparable techno-functionality.

3.2. Introduction

There is concern over the ability of our current food systems to meet the growing protein demand for the rising global population. In light of this, the Food and Agriculture Organization estimates that food production must grow by 25% to 70% in the next 25 years (Hunter et al., 2017). To achieve this growth in production, the food industry is turning to various protein sources. The animal protein industry is expected to continue growing to feed the global population, especially in developing countries (Henchion et al., 2017). However, this is associated with multiple issues such as environmental concern with the production of 12% of direct greenhouse gas emissions (GHG) and 12% indirect GHG; water, air, and soil pollution; intense land and fresh water use for livestock and feed farming; biodiversity loss; and health concerns such as the overconsumption of processed meats high with saturated fatty acids; and the allergenicity of animal protein sources such as eggs and dairy (Berners-Lee et al., 2018; Henchion et al., 2017; Hunter et al., 2017; Ismail et al., 2020).

On the other hand, the plant protein market is also expected to increase due to the food industry realizing the need to address food demand while mitigating the effects of environmental problems. Plant proteins are considered more sustainable due to their lower land use, water use, and GHG emissions. The utilization of novel and sustainable plant protein sources with a good amino acid composition, protein digestibility, nutritional quality, and techno-functional properties in food products is essential in replacing the current traditional protein sources (Can Karaca et al., 2023; Ismail et al., 2020). Sunflower, an oilseed, is gaining interest in the food industry as a source of plant protein. Sunflower is the third major oilseed worldwide, where it is prominently used for oil extraction by pressing or chemical solvents, which results in a sunflower meal (SFM) byproduct high in protein that is usually discarded or used as animal feed (Kaur & Ghoshal, 2022). However, defatted SFM is a promising protein source, containing a range of 30–40% protein on a dry basis, and provides a sustainable approach for utilizing a by-product to produce a protein ingredient for food production to meet the protein demand (Ivanova et al., 2014; Ren et al., 2015). Sunflower meal consists of two main protein fractions of 60–80% 11S saline soluble globulins (helianthinin) and 25–30% 2S water soluble albumins (Kaur & Ghoshal, 2022; Ren et al., 2015). The functionality of these protein fractions, which is vital for plant-based food applications, depends on the pre-treatment of the seeds, extrinsic conditions of the oil extraction, and the protein extraction method (Ivanova et al., 2014; Kaur & Ghoshal, 2022). SFM obtained after harsh industrial oil extraction conditions has reduced solubility, amino acid composition, and functional properties (Kaur & Ghoshal, 2022). Isolating sunflower proteins from SFM using the conventional and industrial-standard alkaline-based extraction method can further limit their applications in food systems. Conventional alkaline extraction–isoelectric precipitation, although widely used for plant-based protein recovery, utilizes harsh chemicals, generates high amounts of wastewater, and

alters protein conformations that promote denaturation and aggregation, thus leading to poor techno-functionality and protein yield (Chandran et al., 2023; Hewage et al., 2022; Ivanova et al., 2012). In addition, alkaline conditions can oxidize sunflower phenolic compounds, especially CGA, into quinones that promote phenolic-protein interactions, thereby increasing phenolic compound co-extraction, imparting an undesirable dark green colour, and reducing techno-functional properties in SFPI (Alexandrino et al., 2017; Kaur & Ghoshal, 2022). In contrast, novel protein extraction methods should be further investigated to better understand their effects on SFPI, as they offer more sustainable alternatives that better preserve protein structure, enhance techno-functional properties, and support efficient large-scale industrial production.

Although SFM is an abundant by-product of the sunflower oil industry, its use as a source of food-grade protein remains limited. This is partly due to the reliance on conventional alkaline extraction methods, which may affect protein structure and functionality, as well as the lack of information on the performance of protein isolates produced from industrially produced SFM. Therefore, further research is needed to evaluate the yield, purity, structural characteristics, and techno-functional properties of sunflower protein isolates (SFPI) obtained from industrial SFM to better understand their techno-functionality and potential application in food systems. This study addresses this gap by assessing the extraction yield, protein purity, techno-functional properties, and structural properties of SFPI produced from SFM.

It is hypothesized that the green and novel extraction technologies (divalent-salt based, monovalent salt-based, and DES) will produce SFPI with enhanced protein purity, recovery, extraction yield than conventional alkaline-based extraction. These alternative extraction methods were also expected to improve or maintain the techno-functional properties of the isolates while better preserving protein structural integrity and limiting extraction-induced denaturation and

aggregation. The objective of the study is to demonstrate the potential of valorizing SFM as an industrial by-product to a functional plant-protein ingredient, thereby supporting waste reduction and promoting a circular economy within global food systems. Furthermore, this study investigates green and efficient extraction technologies to evaluate their potential for producing high-quality SFPI with improved protein purity, extraction yield, techno-functionality, and structural properties. These novel and green methods can be suitable for large-scale industrial production as an alternative to the conventional alkaline-based extraction method.

3.3. Materials and Methods

3.3.1. Raw Materials and Chemicals

The SFM pellets, a by-product of oil extraction at 60 °C, was obtained from Richardson Centre for Food Technology and Research milling facility (Winnipeg, MB, Canada). Glycerol (≥ 95% purity), choline chloride (> 99% purity), sodium chloride, magnesium chloride, sodium hydroxide, hydrogen chloride, and Bradford protein assay kit were purchased from Fisher Scientific (Ottawa, ON, Canada). Laemmli sample buffer, 2-mercaptoethanol, precision plus protein standards (10-250 kDa), Coomassie blue R250, and precast gels (4-15%) were purchased from Bio-Rad Laboratories Ltd. (Mississauga, ON, Canada). All chemicals used were analytical grade.

3.3.2. Milling of Sunflower Meal

The SFM pellets were milled using a Prater-Sterling Impact Mill (M-21, Prater Industries, Bolingbrook, IL, USA) with 0.2 mm screens. The collected SFM flour was stored at -20 °C until further use.

3.3.3. Conventional Alkaline-Based Extraction

The alkaline-based extraction was followed based on the method described by Karaca et al. (2011) with slight modifications. SFM flour was dispersed in distilled water at a 1: 10 (w/v) solid-to-liquid ratio, then homogenized at 10,000 rpm for 1 minute. The dispersion was adjusted to pH 9.5 with 1.0 M NaOH and stirred at 500 rpm for 40 minutes at room temperature. The dispersion was centrifuged at 1,600 ×g for 20 minutes at 4 °C. The pH of the collected supernatant was then adjusted to 4.5 with 1.0 M HCl, and the precipitated protein was isolated through centrifugation at 1,600 ×g for 20 minutes at 4 °C. The pellet was collected and dialyzed at 4 °C for five days using a 3.5 kDa molecular weight cut-off dialysis membrane, with the water being replaced every 4 hours. The dialyzed protein solution was freeze-dried and stored at 4 °C for further analysis.

3.3.4. Monovalent and Divalent Salt-Based Extraction

Monovalent salt-based extractions was conducted based on the method described in Karaca et al. (2011) with slight modifications. The divalent salt-based extraction was conducted based on an optimized method. Monovalent salt-based extraction was conducted using a 0.8 M sodium chloride solution, while the divalent salt-based extraction was conducted using a 0.8 M magnesium chloride and sodium chloride solution (at a 0.75: 0.25 molar ratio, respectively). Firstly, the SFM flour was dispersed in the salt solution at a 1:9 (w/v) solid-to-liquid ratio, then homogenized at 10,000 rpm for 1 minute. The dispersion was stirred for one hour at 200 rpm at room temperature, and then centrifuged at 17,700 ×g for 20 minutes at 4 °C. The obtained supernatant was dialyzed at 4 °C for five days using a 3.5 kDa molecular weight cut-off dialysis membrane, with the water being replaced every 4 hours. The dialyzed protein isolate solution was freeze-dried and stored at 4 °C for further analysis.

3.3.5. Deep Eutectic Solvent Extraction

The DES extraction was carried out following the optimized conditions by Nadeeshani et al. (2026). The DES was synthesized by mixing choline chloride and glycerol at a 1: 2 (w/w) ratio and stirred at 500 rpm at 80 °C until a clear solution was obtained. After the solution is cooled to room temperature, the water content of the DES was adjusted to 54% (w/w). The protein extraction was performed by dispersing SFM flour in the DES at a 1:17.5 (w/w) solid-to-liquid ratio and homogenizing at 10,000 rpm for 1 minute. The dispersion was stirred at 200 rpm for 60 minutes at 50 °C in a circulating water bath fitted with two 3 L jacketed beakers (Model 6200 H7, Fisher Scientific Inc., Pittsburgh, PA, USA). The dispersion was then cooled to room temperature (~25 °C) and centrifuged at 12,000 rpm at 4 °C for 20 minutes. The collected supernatant was dialyzed for 5 days using a 3.5 kDa molecular weight cut-off dialysis membrane, with the water being replaced every 4 hours. The dialyzed protein isolate solution is freeze-dried and stored at 4 °C for further analysis.

3.3.6. Proximate Composition Analysis, Extraction Yield, and Protein Recovery Rate

The crude protein content was analyzed with the Elementar RAPID N Exceed Nitrogen Analyzer (Elementar Ltd., Langenselbold, Germany), with a 5.3 nitrogen conversion factor (Alpiger et al., 2025; Z. Li et al., 2024). The moisture content was analyzed with a conventional air oven (Thermo Fisher Scientific, Pittsburgh, PA, USA) at 105 °C overnight, until a consistent weight was obtained. The crude fat content was analyzed using the Soxhlet method, and the crude ash content was analyzed at 550 °C using a muffle furnace (Model 1100 box furnace, Thermo Scientific, NC, USA). The extraction yield and protein recovery rate of each extraction method were calculated using Equations (1) and (2):

$$Extraction\ yield\ (\%) = \frac{E_p \times P}{R_w} \times 100 \quad (1)$$

$$Protein\ recovery\ rate\ (\%) = \frac{E_p \times P}{R_w \times R_p} \times 100 \quad (2)$$

Where E_p is the weight (g) of extracted protein isolates, P is the extracted protein purity percentage, R_w is the weight (g) of the initial flour sample, and R_p is protein content in initial flour sample.

3.3.7. Characterization of Sunflower Protein isolates

3.3.7.1. Secondary Structure Analysis

Fourier Transform Infrared Spectroscopy (FTIR) (Invenio FTIR spectrometer, Bruker, MA, USA) was used to analyze changes in the secondary structure of extracted SFPI over the 400–4000 cm^{-1} wavenumber range and 4 cm^{-1} resolution, using 120 scans. Origin 2024 software (Origin lab cooperation, MA, USA) was used to further analyze the FTIR spectra using the 2nd derivative, smoothed with the Savitzky-Golay function, and amide I peaks (at 1600–1700 cm^{-1} range) were fitted for identification of corresponding secondary structure components and their proportions (Tintor et al., 2024).

3.3.7.2. Particle Size Distribution

Particle size distribution of SFM flour and extracted SFPI was analyzed using static laser light diffraction (Mastersizer 2000, Malvern Instrument Ltd, Malvern, UK) at a 1.45 refractive index, 0.1 absorption index, and 1.0 particle density. The obtained results are analyzed as volume-weighted mean particle diameter (D [4,3]), surface-area weighted mean particle diameter (D [3,2]), and volume percentiles at Dx (10), Dx (50), and Dx (90).

3.3.7.3. Morphological Characterization

The morphological changes of the extracted SFPI were analyzed using scanning electron microscopy (SEM) (Quanta 650 FEG, FEI Company, OR, USA). Firstly, the SFM flour and SFPI samples were coated with gold-palladium (Au-Pd) with a sputter coating unit (50 mTorr, 45 mA, 60 seconds) and dried under nitrogen purging (Denton vacuum desk II, Denton Vacuum Inc, NJ, USA). The microscopic images were then analyzed using the xT microscope control software (version 6.2.8).

3.3.7.4. Colour Analysis

The colour of the SFM flour and the extracted SFPI was quantitively analyzed using a spectrocolorimeter (LovibondTM LC 100, UK). The sample is placed in a clear cuvette and inserted in the LovibondTM LC 100 aperture for measurement of L*, a*, and b* values on the CIE colour scale.

3.3.8. Techno-Functional Properties of SFPI

3.3.8.1. Solubility

The protein solubility test as influenced by pH was conducted based on the method reported by Karaca et al. (2011) with slight modifications. A 0.5% (w/v) protein isolate solution is prepared in 20 mL of 0.1 M glycine-HCl buffer (pH 3), 0.1 M acetic acid buffer (pH 5), 0.1 M sodium phosphate buffer (pH 7), and 0.1 M Tris buffer (pH 9). The solution is stirred for 1 hour and 30 minutes at 350 rpm at room temperature, then centrifuged at 17,000 ×g for 15 minutes. The protein solubility is measured using the Bradford assay and calculated as a percentage of soluble protein content in the supernatant divided by the total protein content of the sample.

3.3.8.2. Water and Oil Holding Capacity

Percentage of water and oil holding capacity is conducted based on the method of Malomo et al. (2014) with slight modifications. A 10% (w/v) protein isolate concentration is added to 1.5 mL of water or oil in a pre-weighed Eppendorf tube and vortexed for 1 minute. The dispersion is left to rest at room temperature for 30 minutes to facilitate water and oil absorption, then centrifuged at 7,000 ×g for 25 minutes. The supernatant is discarded, and the excess water or oil is drained by placing the Eppendorf tube inverted for 15 minutes. The final weight of the Eppendorf tube is then recorded. The water and oil holding capacity is calculated as one gram of water or oil retained per one gram of protein isolate.

3.3.8.3. Foaming Capacity and Stability

The foaming capacity and stability at different pH levels are determined based on the method adapted from Vogelsang-O'Dwyer et al. (2020). A 1% (w/v) protein dispersion is prepared in 20 mL (V) of buffers at pH 3, 5, 7, and 9, then homogenized for 2 minutes at 17,000 rpm. The height of the resulting foam was measured immediately at 0 minutes (V_0) and after 30 minutes of standing (V_{30}). The percentage of foaming capacity and stability is calculated using Equations (3) and (4):

$$Foaming\ capacity\ (\%) = \frac{V_0}{V} \times 100 \quad (3)$$

$$Foaming\ Stability\ (\%) = \frac{V_{30}}{V_0} \times 100 \quad (4)$$

3.3.8.4. Emulsification Activity and Stability Index

The emulsification activity index (EAI) and emulsification stability index (ESI) are assessed following the method of Karaca et al. (2011) with minor changes. A 10 mL solution was

prepared in a Falcon tube by dispersing the protein at a concentration of 1% (w/v) in pH 3, 5, 7, and 9 buffer solutions. Then, 5 mL of canola oil was mixed into the dispersion and homogenized at 20,000 rpm for 1 minute. A 50 µL aliquot is immediately obtained from the bottom of the tube and diluted in 5 mL of 0.1% sodium dodecyl sulphate (SDS) solution, and the absorbance (A_0) read at 500 nm using a spectrophotometer. The emulsion is left to stand for 10 minutes, after which another 50 µL aliquot from the bottom of the tube is obtained and diluted in 0.1% SDS for absorbance measurement (A_{10}). The EAI and ESI are calculated using Equations (5) and (6):

$$EAI\left(\frac{m^2}{g}\right) = \frac{2 \times 2.303 \times A_0 \times N}{c \times \varphi \times 10{,}000} \tag{5}$$

$$ESI\ (minutes) = \frac{A_0}{\Delta A} \times t \tag{6}$$

Where A_0 is the absorbance of the diluted sample at 0 minutes after homogenization, N is the aliquot dilution factor, c is the weight of the protein per mL of volume, φ is the oil volume fraction in the emulsion, ΔA ($A_0 - A_{10}$) is the change in absorbance between 0 and 10 minutes, and t is the 10-minute time interval between measurements.

3.3.8.5. Least Gelling Capacity

The least gelling capacity of SFM and protein isolated was conducted based on the method described by Vogelsang-O'Dwyer et al. (2020) with minor changes. SFM and protein isolates were dispersed in an Eppendorf tube in 0.1 M acetic acid buffer (pH 5), 0.1 M sodium phosphate buffer (pH 7), and 0.1 M Tris buffer (pH 9) in the range of 1-24% protein weight (w/v) and hydrated at 4 °C for 16 hours. Following hydration, the samples were heated at 90 °C for 1 hour, then cooled immediately in an ice bath and stored at 4 °C overnight. The least gelling capacity was taken at the concentration at which the dispersion inside the inverted tube did not flow or break. The gels were

further characterized as firm when they could be taken out of the tube without breaking, and as soft when they broke when taken out.

3.3.9. Statistical Analysis

Statistical analysis of data was conducted using OriginPro software (version 2024, OriginLab Corporation, MA, USA). All the data were analyzed using Analysis of Variance (ANOVA) with Tukey's test for mean comparisons at a significance level of $P < 0.05$. All analyses were carried out in triplicate.

3.4. Results and Discussion

3.4.1. Proximate Composition Analysis, Extraction Yield, and Protein Recovery Rate

Table 3-1. Proximate composition of sunflower meal and extracted sunflower protein isolates (SFPI).

Sample	Protein purity (%)	Moisture content (%)	Crude fat (%)	Crude ash (%)
SFM	43.45 ± 0.12 ^{C}	5.44 ± 0.25 ^{B}	15.29 ± 0.75 ^{A}	6.85 ± 0.01 ^{A}
DES-SFPI	88.46 ± 1.38 ^{A}	4.56 ± 0.65 ^{BC}	6.68 ± 0.50 ^{BC}	0.67 ± 0.05 ^{C}
DV-SFPI	90.06 ± 1.01 ^{A}	3.29 ± 0.42 ^{C}	6.07 ± 0.12 ^{C}	0.43 ± 0.02 ^{C}
MV-SFPI	90.18 ± 0.48 ^{A}	6.82 ± 0.25 ^{A}	6.29 ± 0.89 ^{C}	1.15 ± 0.07 ^{B}
ALK-SFPI	69.35 ± 0.49 ^{B}	3.58 ± 0.11 ^{C}	9.55 ± 0.71 ^{B}	1.34 ± 0.18 ^{B}

$^{A-C}$ Superscripts denote a significant difference between samples, $P < 0.05$ using Tukey's test.

The proximate composition of SFM and extracted SFPI samples is presented in Table 3-1. The protein content of SFM was found to be 43.45 ± 0.12%, supporting this by-product as a

valuable plant-based protein source. This value is consistent with the reported protein content range of 30–50% for SFM, which can vary depending on the seed dehulling process and the oil extraction conditions (Dauguet et al., 2016; Hadidi et al., 2024; Pickardt et al., 2009). Similar results were reported by Singh et al. (2024), with SFM containing 47.94% protein content. The extraction processes significantly increased the protein purity. The highest protein purity was observed in MV-SFPI (90.18%), followed by DV-SFPI (90.06%), and DES-SFPI (88.46%), with no significant difference between these samples. However, the protein purity of all three samples was significantly higher than that of ALK-SFPI (69.35 $\pm$ 0.49%). This indicates that the DES and salt-based extraction methods are more efficient in concentrating the protein fraction from the source material. The high protein purity of DES-SFPI can be related to the ability of DES systems to improve protein fractionation under milder extraction conditions. Similar findings have been reported for other plant-based proteins, such as rapeseed and lupin, where DES-based extraction produced isolates with high protein purity (Đermanović et al., 2025; Nadeeshani et al., 2026). The protein purities obtained for MV-SFPI, DV-SFPI, and DES-SFPI are comparable to values reported for sunflower protein isolates in previous studies. Zaky et al. (2022) reported an 87.12% protein purity, while Alexandrino et al. (2017) reported 92.68% for SFPI extracted with conventional alkaline-based isoelectric precipitation. These values are higher than the protein content of ALK-SFPI in this study despite using the same extraction methods, where differences may be attributed to variations in raw material, extraction conditions, and freeze-drying parameters. Although alkaline-based extraction is the most common industrial method for plant-based proteins, it poses several challenges for producing SFPI. Alkaline-based extraction can lead to the denaturation of globulin proteins and promote undesirable phenolic-protein interactions causing protein aggregation, resulting in low extractability and protein purity (Beaubier et al., 2021).

The moisture content was highest in MV-SFPI (6.82%), followed by SFM (5.44%) and DES-SFPI (4.56%), while DV-SFPI and ALK-SFPI had the lowest moisture contents, at 3.29% and 3.58%, respectively. The higher moisture content in MV-SFPI may be due to greater water retention by the protein matrix after extraction and drying. The crude fat and ash content of SFM was 15.29% and 6.85%, respectively, which is significantly higher than all SFPI samples. Similar fat (12.04%) and ash (7.95%) contents in SFM were reported by Singh et al. (2024). This high fat content is expected for an oilseed crop such as sunflower, where 1–23.6% residual oil can remain in the meal after oil extraction (Grahovac et al., 2025). All extraction methods significantly reduced the crude fat and ash content compared to SFM. The lowest crude fat content was observed in DV-SFPI (6.07%), followed by MV-SFPI (6.29%) and DES-SFPI (6.68%), with no significant difference between samples. This indicates that DES and salt-based extraction methods are similarly effective in reducing fat content in SFPI. However, ALK-SFPI had the highest fat content among the isolates (9.55%), indicating less efficiency in removing residual fat during conventional alkaline extraction. The lowest ash content was observed in DV-SFPI (0.43%), followed by DES-SFPI (0.67%), while MV-SFPI (1.15%) and ALK-SFPI (1.34%) had significantly higher ash contents among the isolates. The reduction in ash content in the SFPI samples can be due to the removal of minerals and inorganic compounds originally present in SFM during protein extraction and dialysis washing. Higher results were observed by Alexandrino et al. (2017) for alkaline-extracted SFPI with a moisture content of 5.44% and ash content of 2.26%. Conversely, Zaky et al. (2022) observed a significantly lower fat content of 0.32% in alkaline extracted SFPI. As displayed in Table *3-2*, DES-SFPI and MV-SFPI had significantly higher protein recovery rates of 56.92% and 54.12%, respectively, followed by DV-SFPI (45.27%) and ALK-SFPI (24.80%). A similar trend was observed for extraction yield, where DES-SFPI (24.73%) and MV-SFPI

(23.52%) showed the highest values, followed by DV-SFPI (19.67%) and ALK-SFPI (10.78%). This suggests that the DES and monovalent salt-based extraction methods have a higher efficiency in retaining soluble proteins and total extractable solids from SFM. The higher protein recovery of MV-SFPI may be due to the salting-in effect of NaCl, which improves sunflower protein solubility under mild extraction conditions and reduces protein-phenolic interactions (Ivanova et al., 2012; Pickardt et al., 2009). Furthermore, choline chloride-based DES extraction systems were reported as an effective method in extracting plant-based proteins, such as sunflower, due to the disruption of protein-protein interactions and consequent increase of protein-solvent interactions that increase extractability (Karabulut et al., 2025). The significantly low protein recovery rate and extraction yield for ALK-SFPI can be attributed to the high pH conditions that promote protein denaturation, aggregation, and protein-phenolic interactions, thus reducing protein recovery during precipitation and washing and having a lower retention of soluble proteins in the final isolate.

Table 3-2. Protein recovery rate, and extraction yield of sunflower meal (SFM) and extracted sunflower protein isolates (SFPI).

Sample	Extraction yield (%)	Protein recovery rate (%)
DES-SFPI	24.73 ± 0.50^{A}	56.92 ± 0.61^{A}
DV-SFPI	19.67 ± 0.29^{B}	45.27 ± 1.80^{B}
MV-SFPI	23.52 ± 0.78^{A}	54.12 ± 1.15^{A}
ALK-SFPI	10.78 ± 0.27^{C}	24.80 ± 0.66^{C}

$^{A-C}$ Superscripts denote a significant difference between samples, $P < 0.05$ using Tukey's test.

3.4.2. Characterization of Sunflower Protein Isolates

3.4.2.1. Secondary Structure Analysis

The secondary structure changes of SFM and SFPI samples, as presented in Figure *1*, were analyzed by deconvoluting the amide I region (1600–1700 cm^{-1}) of the FTIR spectra, which arises from the C=O bond stretching vibrations associated with the protein secondary structures. Second-derivative analysis was performed to deconvolute constituent bands, followed by Gaussian peak fitting to obtain individual secondary structure components. The fitted peak positions for individual secondary structure components were assigned based on published data (Tintor et al., 2024). The relative areas of the fitted peaks were used to quantify the contributions of β-turns, α-helix, β-sheets, and random coils are summarized in Table *3-3*.

It can be seen in Figure *1*a that all SFPI samples had prominent amide I and II peaks, while the FTIR spectrum of SFM had less pronounced peaks, indicating a lower protein fraction in the meal matrix. In contrast, the prominent absorption peak around 1000 cm^{-1} for SFM is associated with C-O-C stretching vibrations of non-starch polysaccharide content, while its reduction in the isolates confirms the enrichment of protein after extraction (Liu et al., 2021). Across all samples, β-sheet structures were the dominant secondary structure, ranging from 46.67% in DES-SFPI to 57.13% in SFM. This is consistent with Li et al. (2024), in which sunflower meal proteins from different varieties were reported to be mainly composed of β-sheet structures, although the β-sheet content of 57.13% in SFM found in this study is higher than their reported range of 25.56–47.15%. This difference may be due to the sunflower variety, oil processing, residual phenolics and carbohydrates, or differences in spectral deconvolution. The high β-sheet content in SFM suggests a compact, ordered protein structure, whereas the reduction in SFPI may reflect partial disruption of the compact protein network during extraction. DV-SFPI retained the highest β-sheet content

among the isolates (52.93%) and showed the highest α-helix content (36.74%), with no detectable random coil. This suggests that divalent salt-based extraction may have better preserved ordered protein conformations. Moreover, the small side-chain contribution detected only in DV-SFPI may indicate exposure or reorientation of amino acid side groups during extraction. MV-SFPI and DES-SFPI showed lower β-sheet contents of 49.51% and 46.67%, respectively, with the presence of random coil structures. DES-SFPI had the highest random coil content (10.76%), suggesting that this extraction method induced partial unfolding of ordered structures by modifying protein hydrogen bonding and backbone conformation (Karabulut et al., 2025). Conversely, Karabulut et al. (2025) observed that DES-extracted SFPI showed more preserved β-sheet structures in the amide I region than the results in this study. This difference may be attributed to variations in DES constituents and composition, water content, and extraction temperature. On the other hand, ALK-SFPI showed a distinct structural profile, with a comparable β-sheet content (46.95%), the lowest α-helix content (27.56%), and the highest β-turn content (17.83%). This indicates that alkaline extraction caused a greater rearrangement and unfolding of the protein backbone than salt or DES extraction. During alkaline extraction, the high pH can alter protein structure and promote aggregation. Subsequent adjustment to an acidic pH at the isoelectric point neutralizes the protein surface charge, thus increasing the hydrophobic interactions and further promoting protein aggregation (Beaubier et al., 2021; X. Huang et al., 2024; Patel et al., 2025).

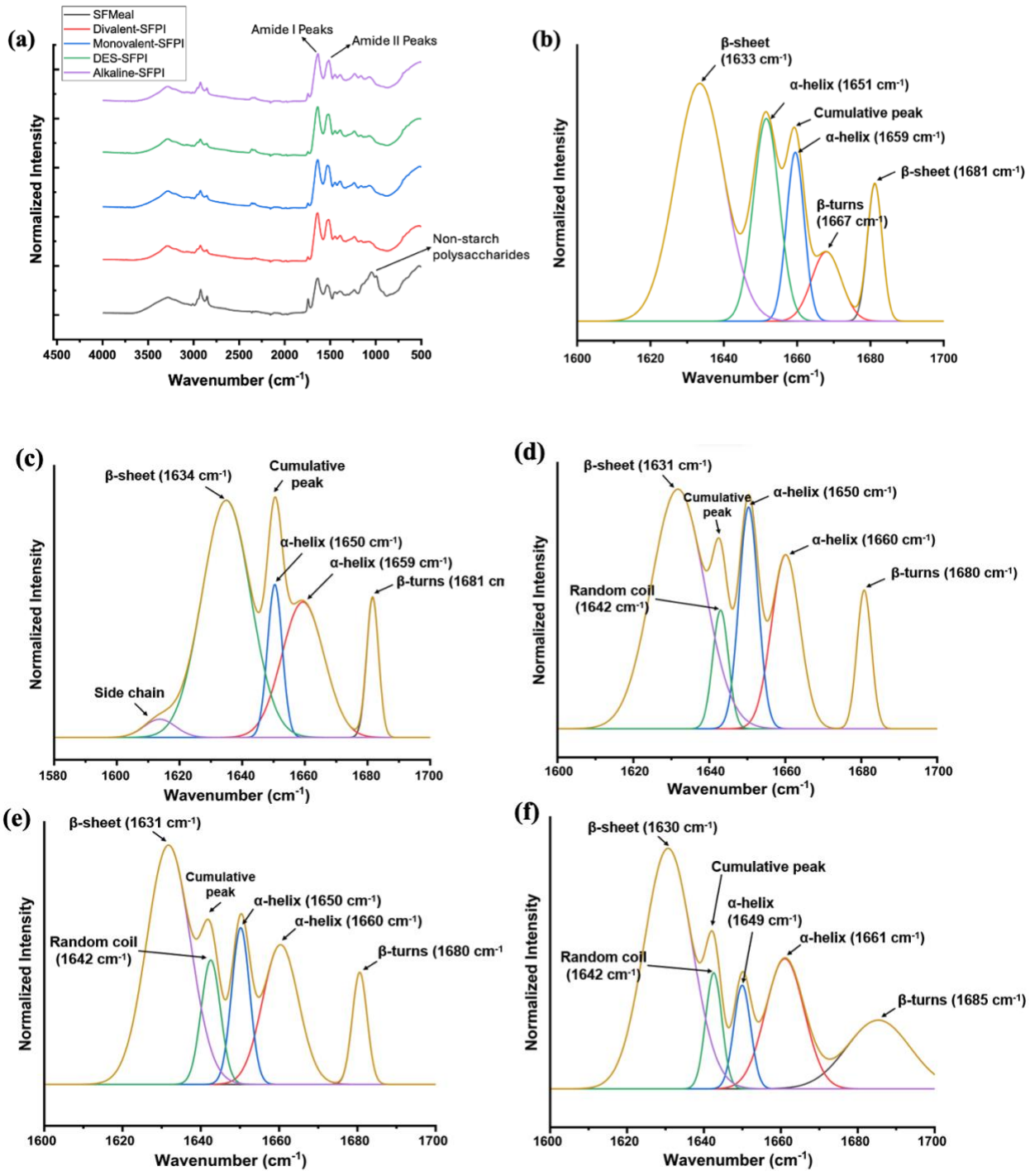

Figure 1. Secondary structures of sunflower meal (SFM) and extracted sunflower protein isolates (SFPI). (a) Amide I and II spectra, (b) SFM, (c) Divalent salt-based sunflower protein isolate (DV-SFPI), (d) Monovalent salt-based sunflower protein isolate (MV-SFPI), (e) DES-extracted

sunflower protein isolate (DES-SFPI), (f) Alkaline extracted sunflower protein isolates (ALK-SFPI).

Table 3-3. Relative proportions of protein secondary structures for sunflower meal flour (SFM) and extracted sunflower protein isolate (SFPI) samples.

Sample	Secondary structure relative content (%)				
	Side chains	α-helix	β-turns	β-sheets	Random coil
SFM	-	34.34	8.54	57.13	-
DV-SFPI	2.63	36.74	7.71	52.93	-
MV-SFPI	-	35.50	7.77	49.51	7.21
DES-SFPI	-	35.12	7.45	46.67	10.76
ALK-SFPI	-	27.56	17.83	46.95	7.66

3.4.2.2. Particle Size Distribution

The particle size distributions of the SFM and SFPI samples are shown in Figure *2*, and the corresponding mean particle diameters are presented in Table *3-4*. Overall, the extraction methods had clear differences in their distributions, indicating that the conditions used to isolate the protein influence the particle breakdown, dispersibility, and aggregation. SFM displayed a higher D [4,3] value of 71.50 μm than the D [3,2] value of 15.30 μm, indicating a broad particle size distribution containing both large and small particles. SFM had 50% of particles being smaller than 38.37 μm and 90% were smaller than 137.33 μm. The significantly low SFM volume percentile values may result from particle size reduction during milling of defatted sunflower pellets into SFM flour. This is vital for protein extraction, as it can facilitate efficient solvent penetration and protein

solubilization from the cell matrix. The SFM milled in this study was much finer than sunflower meal flour reported in previous studies. Vidosavljević et al. (2022) reported that more than 50% of sunflower meal particles were larger than 1250 μm. This discrepancy may be due to differences in milling conditions, defatting process, and the starting meal structure. Among the extracted isolates, DES-SFPI showed significantly lower values across all particle size distribution parameters, except D [4,3], and was the closest to SFM. DES-SFPI had a D [4,3] value of 85.37 μm, D [3,2] of 20.03 μm, Dx (50) of 41.57 μm, and Dx (90) of 151.33 μm. This indicates that DES extraction produced finer particles compared to other extraction methods. This can be attributed to mild extraction conditions, reduced protein aggregation, and increased protein solubilization via hydrogen bonding in DES systems (Hewage et al., 2024; Karabulut et al., 2025; Nadeeshani et al., 2026). Karabulut et al. (2025) reported that DES-extracted SFPI produced smooth, spherical particles with a particle size of 222.6 nm. This value is much smaller than the DES-SFPI obtained in the present study, where the difference may be caused by the measurement conditions. DV-SFPI and MV-SFPI showed a broader particle size distribution, which was significantly wider than that of SFM and DES-SFPI. The larger particle size of DV-SFPI and MV-SFPI may be related to the effects of divalent and monovalent salt ions during extraction, which reduce protein electrostatic repulsion and promote the formation of ionic bridges between negatively charged groups on the protein surface, thereby promoting mild protein aggregation (Bryant & McClements, 2000). ALK-SFPI showed the largest particle size among all samples with the highest D [4,3], D [3,2], Dx (90), Dx (50), and Dx (10) values of 121.00 μm, 50.73 μm, 261.67 μm, 93.27 μm, and 22.97 μm, respectively.

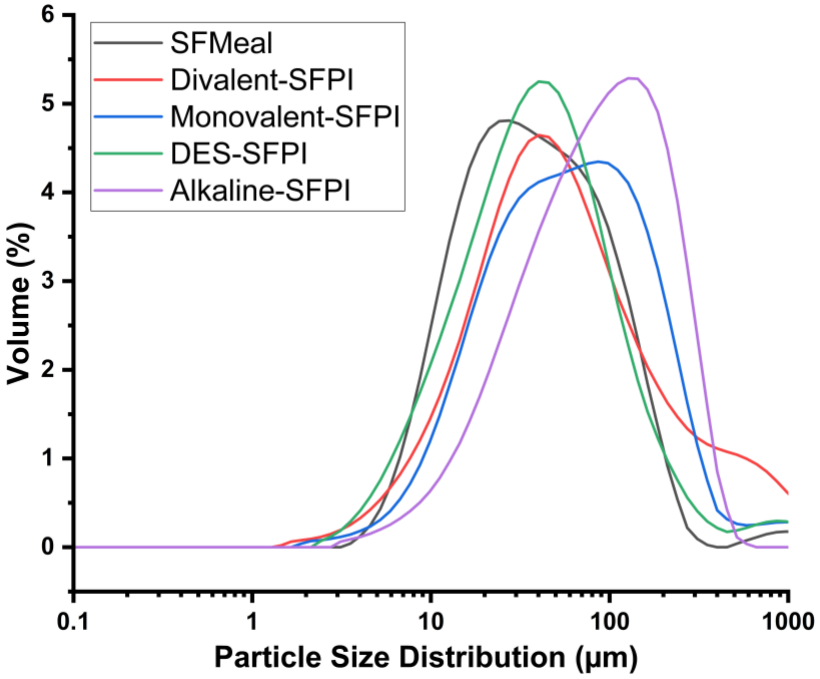

Figure 2. Particle size distribution of sunflower meal (SFM) and extracted sunflower protein isolates (SFPI).

Table 3-4. Mean particle diameter values of sunflower meal (SFM) and extracted sunflower protein isolates (SFPI).

Sample	D [4,3]	D [3,2]	Dx (90)	Dx (50)	Dx (10)
SFM	71.50 ± 16.26 ^{A}	15.30 ± 0.80 ^{E}	137.33 ± 11.39 ^{B}	38.37 ± 1.05 ^{D}	11.06 ± 0.10 ^{D}
DES-SFPI	85.37 ± 14.67 ^{A}	20.03 ± 1.07 ^{D}	151.33 ± 11.98 ^{B}	41.57 ± 0.84 ^{D}	10.97 ± 0.15 ^{D}
DV-SFPI	107.90 ± 13.73 ^{A}	28.27 ± 0.99 ^{C}	221.33 ± 22.24 ^{A}	51.80 ± 1.15 ^{C}	13.13 ± 0.19 ^{C}
MV-SFPI	100.47 ± 10.40 ^{A}	36.00 ± 0.67 ^{B}	223.00 ± 15.13 ^{A}	63.27 ± 1.17 ^{B}	16.10 ± 0.27 ^{B}
ALK-SFPI	121.00 ± 3.51 ^{A}	50.73 ± 0.63 ^{A}	261.67 ± 8.88 ^{A}	93.27 ± 1.28 ^{A}	22.97 ± 0.23 ^{A}

$^{A-C}$ Superscripts denote a significant difference between samples, $P < 0.05$ using Tukey's test.

The particle size distribution curve (Figure *2*) also showed a clear rightward shift, with the main peak occurring at a larger particle size than in the other samples. This indicates an increase in protein unfolding during alkaline conditions, causing the exposure of hydrophobic groups that reassociate and form large aggregates (Salgado et al., 2012; Yao et al., 2023). The high D [4,3] and D [3,2] values suggest that the increase in particle size was caused by an overall reduction in fine particles and increase in coarser particles. Furthermore, alkaline extraction promotes protein-phenolic interactions that form complexes, producing larger aggregates in ALK-SFPI (Karefyllakis et al., 2017; Malik et al., 2016). The particle size differences observed in this study may influence the functional properties of the isolates, where smaller particles with a larger surface area can improve solubility, emulsification, and foaming capacity (Malik et al., 2017).

3.4.2.3. Morphological Characterization

SEM imaging, displayed in Figure *3*, was used to evaluate the surface morphology and particle organization of SFM and SFPI samples produced with four extraction methods. The images show clear differences among samples, indicating that the extraction conditions influence the physical structure of the isolates. The irregular agglomerated particles with surface fissures found in SFM (Figure *3*a) may represent the disruption of the sunflower meal matrix following oil extraction and milling, while the small globular structures can be identified as protein storage bodies or starch granules (Z. Li et al., 2024; Liu et al., 2021). After protein extraction, the SFPI particles appeared as irregular sheet-like fragments and small granular aggregates. This can be due to structural rearrangement during protein extraction and the effect of freeze-drying. A similar freeze-drying effect on other plant-based proteins, such as lupin, fava bean, and soybean, was observed in previous studies (Hewage et al., 2024; Nadeeshani et al., 2026; Wang et al., 2020).

DV-SFPI had more granular and aggregated small protein-rich particles compared with MV-SFPI, which exhibited larger plate-like fragments and fewer densely aggregated granular particles.

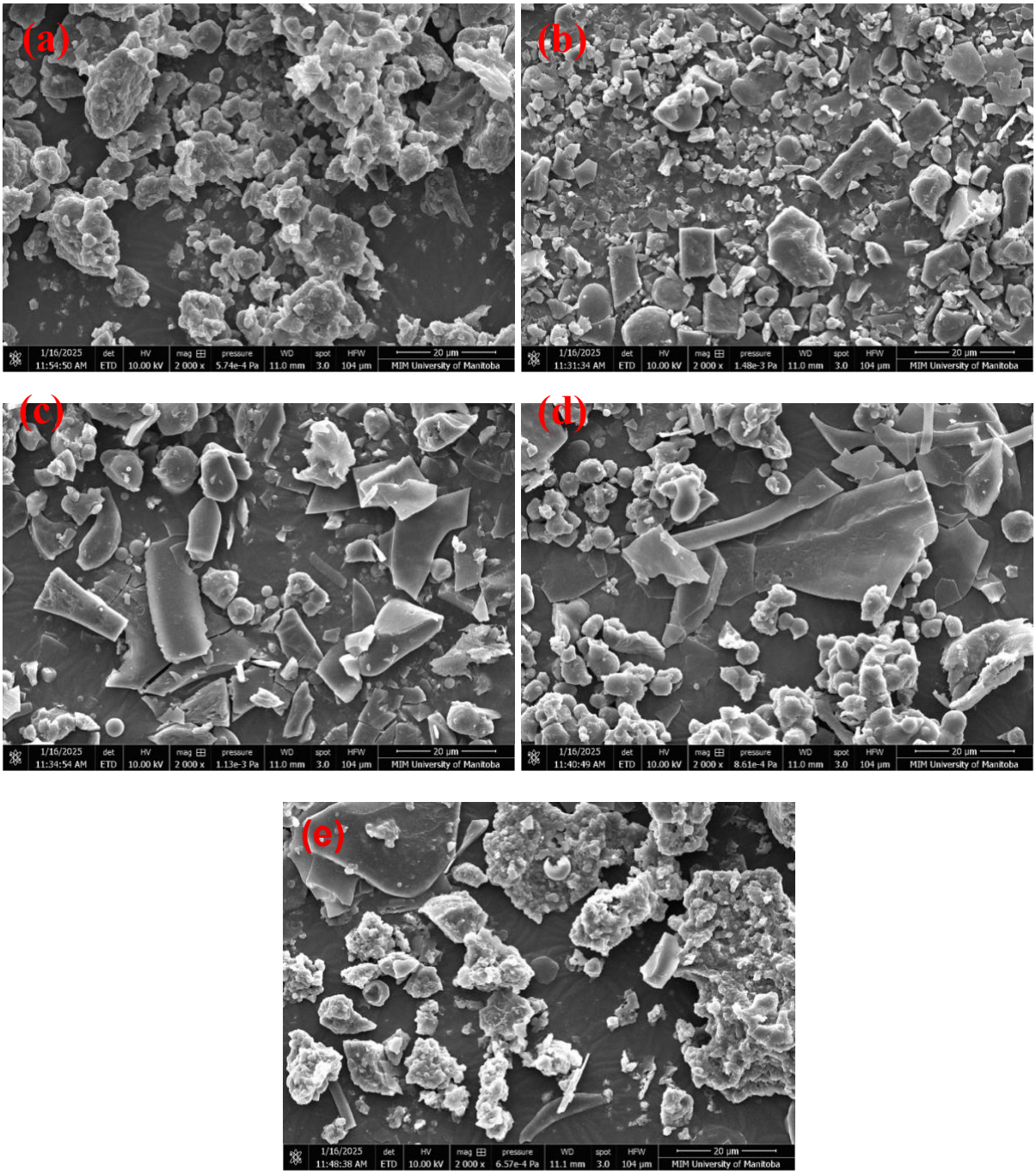

Figure 3. Scanning electron microscopy (SEM) imaging of (a) Sunflower meal (SFM), (b) Divalent salt-based sunflower protein isolate (DV-SFPI), (c) Monovalent salt-based sunflower protein

isolate (MV-SFPI), (d) DES-extracted sunflower protein isolate (DES-SFPI), (e) Alkaline extracted sunflower protein isolates (ALK-SFPI).

The increased aggregation in DV-SFPI may result from divalent cations' ability to bridge negatively charged protein groups more effectively than monovalent ions, thereby promoting stronger protein-protein interactions during extraction or drying. DES-SFPI showed a similar morphology to MV-SFPI, while ALK-SFPI displayed large, irregular, rough-surfaced aggregates. This morphology indicates stronger protein aggregation and structural disruption during alkaline extraction and isoelectric precipitation. This is consistent with previous studies that also found alkaline extracted SFPI formed irregular aggregates, while milder extraction approaches produced smoother or more continuous particle structures (X. Huang et al., 2024; Karabulut et al., 2025).

3.4.2.4. Colour Analysis

Table 3-5. Qualitative colour analysis on the CIE colour scale for sunflower meal and sunflower protein isolate samples.

Sample	L*	a*	b*	C*	h*
SFM	77.67 ± 0.73^{B}	1.63 ± 0.07^{B}	15.37 ± 0.03^{A}	15.43 ± 0.03^{A}	83.90 ± 0.35^{A}
DES-SFPI	78.03 ± 0.19^{B}	1.77 ± 0.03^{AB}	15.43 ± 0.15^{A}	15.53 ± 0.15^{A}	83.53 ± 0.12^{A}
DV-SFPI	84.07 ± 0.23^{A}	0.97 ± 0.03^{C}	15.30 ± 0.15^{A}	15.33 ± 0.13^{A}	86.33 ± 0.07^{A}
MV-SFPI	68.63 ± 0.12^{C}	1.87 ± 0.03^{A}	15.47 ± 0.33^{A}	15.57 ± 0.33^{A}	83.00 ± 0.06^{A}
ALK-SFPI	65.77 ± 0.34^{D}	-1.13 ± 0.03^{D}	14.37 ± 0.62^{A}	14.37 ± 0.62^{A}	94.63 ± 0.07^{A}

$^{A-D}$ Superscripts denote a significant difference between samples, $P < 0.05$ using Tukey's test.

The qualitative CIE colour scale parameters of the SFM and SFPI samples are presented in Table *3-5*, with corresponding visual representations shown in Figure 4. Significant differences in the lightness (L*) of the samples were observed, indicating that the extraction method influences on this parameter. DV-SFPI was the lightest sample with the highest L* value of 84.07 ± 0.23, suggesting that the divalent salt-based extraction conditions may reduce phenolic-protein interactions and the formation of dark phenolic compounds. The second lightest sample was DES-SFPI, with an L* value of 78.03 ± 0.19, with no significant difference in lightness compared to SFM. In contrast, ALK-SFPI had the lowest L* value (65.77 ± 0.34), followed by MV-SFPI (68.63 ± 0.12) which were both significantly darker than the SFM raw material and the DES-SFPI and DV-SFPI samples. The a* (positive red to negative green dimension) values also differed significantly between samples. SFM, DES-SFPI, DV-SFPI, and MV-SFPI all had positive a* values, indicating a slight reddish-brown hue, with MV-SFPI and DES-SFPI having the highest positive values of 1.87 ± 0.03 and 1.77 ± 0.03, respectively. DV-SFPI had the lowest positive a* value of 0.97 ± 0.03 where it exhibits the least reddish-brown hue between samples. Conversely, ALK-SFPI had a negative a* value (-1.13 ± 0.34), reflecting a green hue in the isolate. This is commonly reported in SFPI samples extracted under alkaline conditions, which is very undesirable for food applications (Alexandrino et al., 2017; Saeed & Cheryan, 1988; Salgado et al., 2011). There were no significant differences in the b* values (positive yellow to negative blue dimension), ranging from 14.37 ± 0.62 to 15.47 ± 0.33, denoting that all samples had a yellow component. Similarly, C* values and h* were not significantly different among samples, indicating that the colour saturation and hue angle were similar despite differences in extraction method. Salgado et al. (2011) reported that SFPI samples produced by alkaline-based extraction, without prior removal of phenolic compounds, exhibited a green colour as reflected by the negative a* value, and a dark

colour with a low L*. Similarly, Alexandrino et al. (2017) reported that alkaline extracted SFPI had a green hue with a negative a* value of -7.02 $\pm$ 0.03, and a dark colour with an L* value of 51.66 $\pm$ 0.12. Both values were lower than those observed in this study, with the SFPI samples having a lighter and less green appearance.

Although CGA is known to contribute to the development of a dark green colour through its interactions with proteins during extraction, the colour parameters measured in this study cannot be directly correlated with the phenolic content results observed in Section 4.3.2.3. A similar lack of direct correlation between the colour analysis and the phenolic content was also reported in other papers (Alexandrino et al., 2017; Salgado et al., 2011). Despite the phenolic content in the samples, the extraction method conditions play a major role in determining the colour of SPFI. The alkaline conditions during the conventional isoelectric precipitation extraction induce the oxidation of CGA to o-quinone compounds that interact with the proteins, leading to the formation of a dark green and brown colour (Salgado et al., 2011). Therefore, extraction methods that do not have an alkaline environment reduce phenolic oxidation and subsequent protein-phenolic interactions.

Figure 4. Sample picture displaying colour difference in sunflower protein isolates.

3.4.3. Techno-Functional Properties

3.4.3.1. Solubility

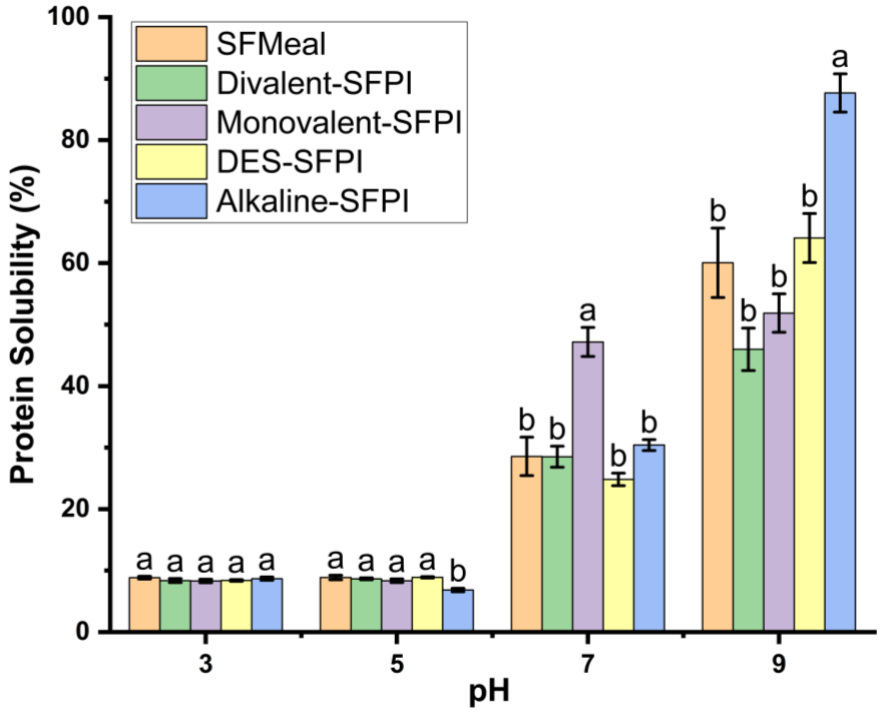

Figure 5. Protein solubility of sunflower meal and sunflower protein isolates in different pH conditions.

The solubility of SFM and SFPI, shown in Figure *5*, was evaluated at pH 3, 5, 7, and 9. Overall, protein solubility was strongly pH-dependent, which is consistent with previous studies on sunflower proteins that report low solubility around the isoelectric point and improved solubility at higher pH values due to increased protein net charge and electrostatic repulsion (Beaubier et al., 2021; X. Huang et al., 2024; Subaşı et al., 2020). At pH 3, all samples had low protein solubility, ranging from 8.32% to 8.85%, with no significant differences among the treatments. This may be due to the formation of aggregates caused by the acidic pH close or lower than the isoelectric point, thereby limiting protein hydration (Muhammad et al., 2026; Subaşı et

al., 2020). Similarly, the low solubility ranging from 8.36–8.92% at pH 5 is expected, as it is the isoelectric region of the main sunflower protein fraction, 11S globulins known as helianthinin, where they exhibit minimum solubility at pH 4–6 (Beaubier et al., 2021; González-Pérez et al., 2002; Z. Li et al., 2024). Similarly, Malik & Saini (2017) reported SFPI samples exhibiting minimum solubility at pH 5. ALK-SFPI showed a significantly lower solubility of 6.83 ± 0.28% at pH 5. This suggests that alkaline extraction may have produced proteins that were more prone to precipitation or aggregation around the isoelectric point. At pH 7, MV-SFPI had the highest solubility of 47.19 ± 2.37% and was significantly more soluble than all other samples. This may be attributed to the ability of monovalent salt-based extraction to reduce the formation of insoluble aggregates and recover soluble sunflower protein fractions, particularly albumins, which are highly water-soluble and contribute to overall protein solubility (Beaubier et al., 2022; Sara et al., 2020). Alexandrino et al. (2017) reported a high protein solubility (96.66%) at pH 7 for alkaline-extracted SFPI, compared to the value of 30.43% found in this study. In contrast, the results observed in this study agree with Huang et al. (2024), who reported that sunflower protein concentrates produced with alkaline extraction had a reduced solubility compared to other extraction methods such as ultrafiltration. Conversely, all SFPI samples exhibited the highest solubility at pH 9, with ALK-SFPI having a significantly higher solubility of 87.67 ± 3.11%. This can be due to the increased negative charge on protein molecules under alkaline conditions, which promotes electrostatic repulsion between molecules, thus reducing aggregation and improving solubilization (Beaubier et al., 2021; X. Huang et al., 2024). A similar effect was observed by Muhammad et al. (2026), where solubility for ultrasonic-assisted extracted SFPI increased from 5.1 ± 0.9% at pH 4 to 47.0 ± 1.3% at pH 9. From a food application perspective, MV-SFPI is the most suitable for food

formulation such as beverages, emulsions, and foams as they are most produced at neutral pH rather than acidic and alkaline conditions.

3.4.3.2. Water and Oil Holding Capacity

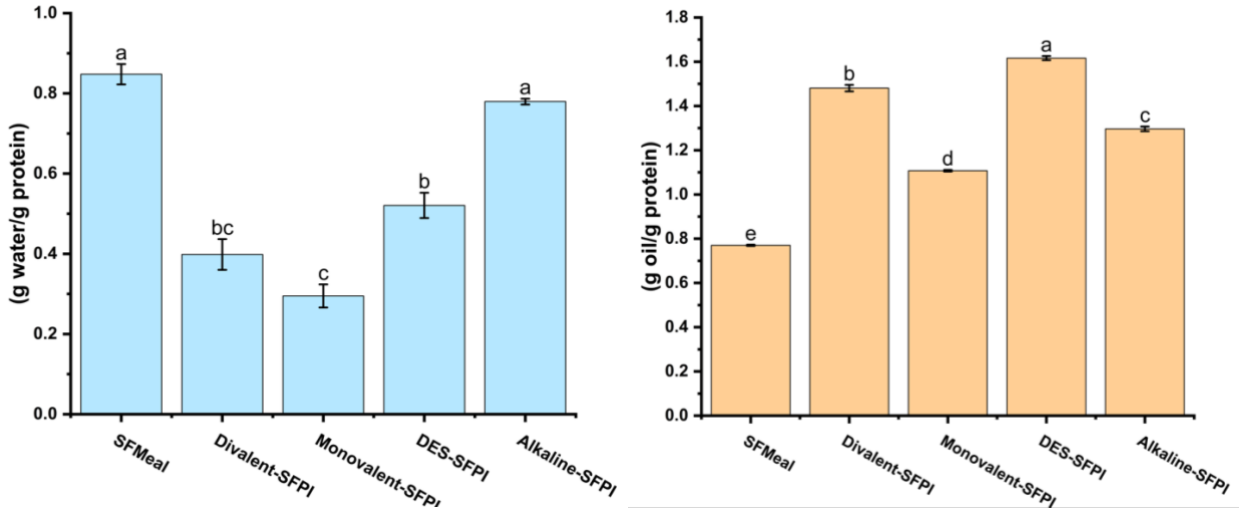

Figure 6. Water holding capacity (a) and oil holding capacity (b) of sunflower meal and sunflower protein isolate samples.

WHC differed significantly among SFM and SFPI samples. SFM showed the highest WHC of 0.85 ± 0.03 g water/g protein and was statistically similar to ALK-SFPI with 0.78 ± 0.01 g/g. DES-SFPI showed an intermediate WHC (0.52 ± 0.03 g/g), followed by DV-SFPI (0.40 ± 0.04 g/g), and MV-SFPI with the lowest value at 0.30 ± 0.03 g/g. These results indicate that SFM and ALK-SFPI had the greatest ability to retain water. The high WHC of SFM may be due to the presence of 29–52% carbohydrates, such as non-starch polysaccharides, that contribute to water retention (Düsterhöft et al., 1992; Liu et al., 2021). Moreover, alkaline extraction can promote protein unfolding and expose polar groups that increase water retention. The WHC values for SFPI found in this study (ranging from 0.78–0.30 g/g) are lower than the range of 1.17–2.11 g/g reported by Li et al. (2024), and the range of 3.20–3.50 g/g reported by Huang et al. (2024). This

discrepancy may be attributed to differences in sunflower variety, protein extraction methods, amino acid composition, and protein structural conformations. The moderate WHC of DES-SFPI, compared to DV-SFPI and MV-SFPI, may be associated with changes in protein conformation caused by DES solvent-protein interactions, which can modify hydrogen bonding, protein structure, and hydrophilicity (Karabulut et al., 2025).

All SFPI samples had significantly higher OHC than SFM. SFM had the lowest OHC of 0.77 ± 0.003 g oil/g protein. In contrast, DES-SFPI showed the highest OHC, with a value of 1.62 ± 0.01 g/g, followed by DV-SFPI (1.48 ± 0.01 g/g), ALK-SFPI (1.30 ± 0.01 g/g), and MV-SFPI (1.11 ± 0.004 g/g). This demonstrates that protein isolation improved OHC compared with SFM, likely because the extraction methods increased the exposure of hydrophobic sites that can interact with lipid molecules. The protein conformational changes caused by interactions with the DES system may have exposed more hydrophobic groups with non-polar amino acid side chains that can bind to lipids more readily than other SFPI samples. Furthermore, the second highest OHC value found in DV-SFPI may be related to the ability of divalent ions to promote protein-protein interactions, which can expose hydrophobic regions within the protein matrix and enhance oil retention. Compared with previous sunflower protein studies, the OHC values in the present study were generally lower. Muhammad et al. (2026) OHC value of 2.90 ± 0.20 g/g for ultrasound-assisted SFPI. On the other hand, Li et al. (2024) reported a comparable OHC range of 1.78–2.14 g/g for sunflower meal proteins from different varieties.

3.4.3.3. Foaming Capacity and Stability

Foaming capacity varied significantly among SFM and SFPI samples, and the effect of the extraction method on foaming techno-functionality was pH dependent. Overall, SFM exhibited the lowest foaming capacity at all pH levels. At pH 3, SFM had a foaming capacity of 13.33 ± 0.83%,

whereas DV-SFPI, DES-SFPI, and MV-SFPI showed much higher values with similar significance of 58.33 ± 7.95%, 55.00 ± 1.44%, and 51.67 ± 0.83%, respectively. ALK-SFPI showed a significantly lower foaming capacity (28.33 ± 2.21%) than the other isolates. A similar trend was observed at pH 5, where DV-SFPI showed the highest foaming capacity (77.50 ± 9.46%), followed by MV-SFPI at 56.66 ± 2.21%, and DES-SFPI at 48.33 ± 6.67%. ALK-SFPI showed a lower value of 25.83 ± 0.83%, while SFM had the lowest foaming capacity of 7.50 ± 0.00%. The high foaming capacity of DV-SFPI at pH 5 suggests that the divalent extraction method produced proteins that were effective at air incorporation near the isoelectric point. SFPI samples had a reduced foaming capacity at pH levels above the isoelectric point. At pH 7, DV-SFPI and DES-SFPI had the significantly highest foaming capacities of 45.83 ± 0.83% and 40.00 ± 0.00%, respectively. ALK-SFPI showed an intermediate value of 29.16 ± 3.33%, while MV-SFPI had a lower value of 25.00 ± 1.44%. SFM again showed the lowest foaming capacity at 18.33 ± 2.21%. These results suggest that DV-SFPI and DES-SFPI may have had more favorable interfacial properties at neutral pH than MV-SFPI and ALK-SFPI, which is important as pH 7 is highly relevant to many food systems. Although MV-SFPI showed the highest solubility at neutral pH, its foaming capacity at pH 7 was not the highest, likely due to the inability of the proteins to rapidly unfold and adsorb into the foaming interface. pH 9 exhibited the lowest foaming capacity, with no significant difference between the SFPI samples. ALK-SFPI had the highest value at 30.83 ± 2.21%, followed by DV-SFPI at 29.17 ± 3.63%, MV-SFPI at 27.50 ± 2.50%, and DES-SFPI at 24.17 ± 3.01%. SFM remained the lowest at 16.67 ± 1.67%. The relatively improved foaming capacity of ALK-SFPI at pH 9 may be related to its high solubility under alkaline conditions.

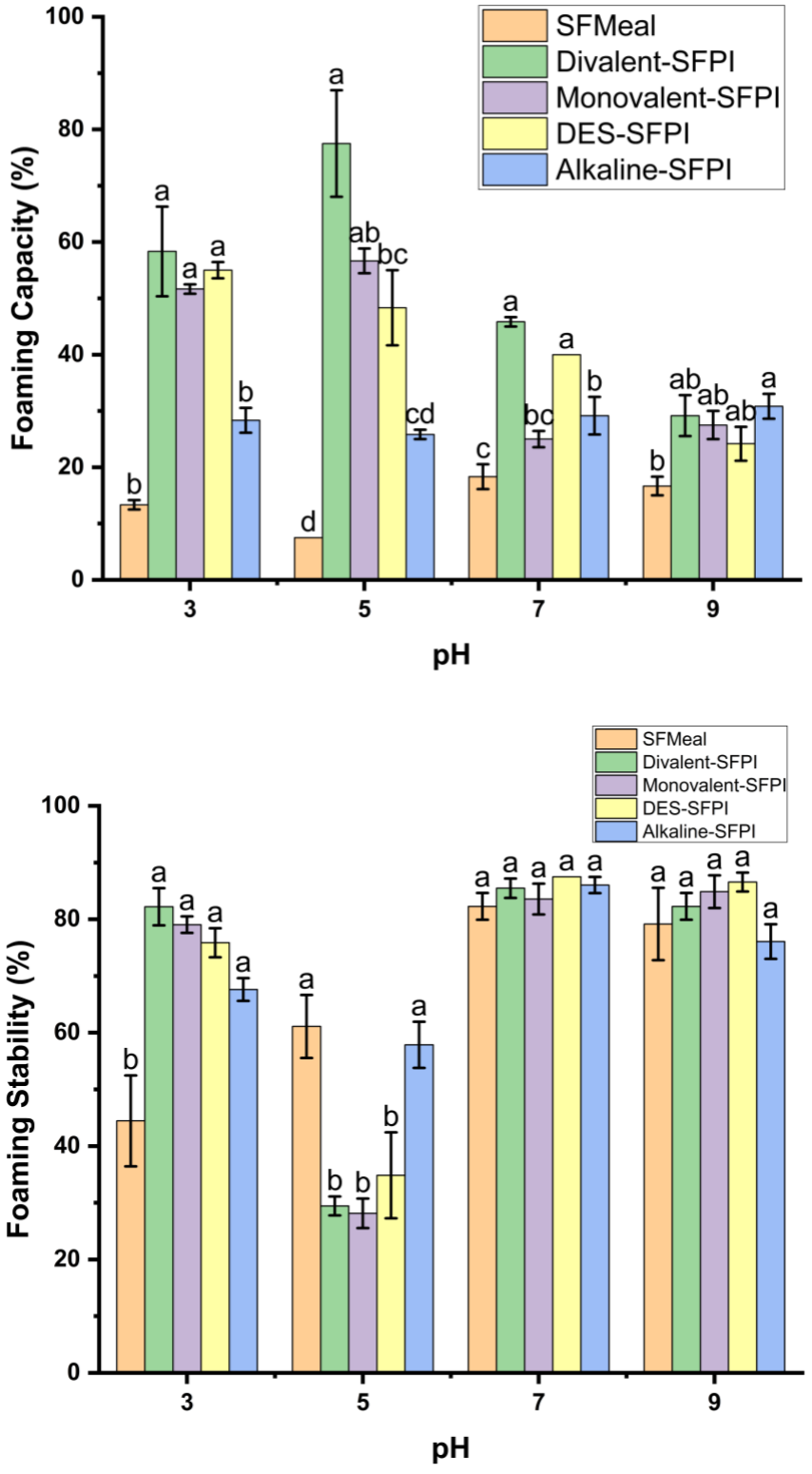

Figure 7. Foaming capacity and stability of sunflower meal and sunflower protein isolate samples at different pH conditions.

At alkaline pH levels, sunflower proteins carry a negative charge that increases electrostatic repulsion, thus limiting protein-protein interactions at the air-water interface and reducing foaming (X. Huang et al., 2024; Muhammad et al., 2026).

Foaming stability was less variable among samples than foaming capacity. At pH 3, SFM had the lowest foaming stability of 44.44 ± 8.01%, while the SFPI samples had no significant difference with values ranging from 82.21–67.62%. Conversely, foam stability at pH 5 decreased sharply for DV-SFPI, MV-SFPI, and DES-SFPI, with values ranging from 29.43–34.84%, whereas SFM and ALK-SFPI maintained a significantly higher stability at 61.11 ± 5.56% and 57.87 ± 4.08%, respectively. At pH 7 and 9, all samples showed high foam stability, with no significant differences between samples, ranging from 82.28–87.50% at pH 7 and 76.08–86.57% at pH 9.

Li et al. (2024) reported foaming capacities ranging from 25.5 to 50.9% and foam stability values from 11.79 to 20.08% for sunflower seed meal proteins from different varieties, which is considerably lower than values observed in this study. Muhammad et al. (2026) observed a higher foaming capacity and stability at acidic pH levels, with values of 45.7 ± 1.0% and 30.9 ± 0.8%, respectively. At pH 7, their foaming capacity and stability decreased to 36.7 ± 0.1% and 9.3 ± 0.4%, and at pH 9 they decreased further to 24.1 ± 0.3% and 4.4 ± 0.1%. A similar effect was seen in this study with a comparable or higher foaming capacity at acidic pH levels, but a higher foaming stability at alkaline pH levels. These results indicate that protein extraction improved the sunflower proteins' ability to form foams compared with original SFM matrix. This improvement may be due to the increased protein concentration with enhanced diffusion and adsorption at the air-water interface during foam formation (Rodriguezpatino et al., 2007). Moreover, the excessive protein aggregation of ALK-SFPI and phenolic-protein interactions can reduce foaming by limiting protein diffusion to the air-water interface (Subaşı et al., 2020).

3.4.3.4. Emulsification Activity and Stability Index

The differences observed among SFM and SFPI samples can be attributed to the effects of pH and extraction method on protein dispersion, interfacial adsorption, and emulsion stabilization. At pH 3, the SFPI samples had a significantly higher emulsification activity index (EAI) than SFM. SFM had the lowest value of 1.59 ± 0.19 m^2/g, whereas DV-SFPI showed the highest EAI at 9.11 ± 0.33 m^2/g, followed by MV-SFPI, DES-SFPI, and ALK-SFPI at 8.49 ± 0.54, 7.46 ± 0.09, and 7.16 ± 0.38 m^2/g, respectively. This indicates that protein extraction improved the ability of sunflower proteins to form emulsions under acidic conditions, which may be related to the increase in surface-active proteins in SFPI that can adsorb at the oil-water interface during homogenization (Damodaran, 2005; Z. Li et al., 2024). At pH 5, MV-SFPI had the highest EAI of 7.22 ± 0.08 m^2/g, followed by DES-SFPI (6.16 ± 0.28 m^2/g), ALK-SFPI (5.54 ± 0.20 m^2/g), DV-SFPI (5.46 ± 0.26 m^2/g), and SFM with the lowest value at 5.18 ± 0.03 m^2/g. The lower EAI for SFM at the acidic pH and isoelectric point may be due to its reduced solubility and the interference of non-protein components within the meal matrix such as carbohydrates (non-starch polysaccharides), fibre, and lipids which can limit the availability of proteins and compete for adsorption at the oil-water interface (Kaczmarek et al., 2025; K. K. Ma et al., 2022). At pH 7, SFM and ALK-SFPI showed the highest EAI values with no significant difference of 10.55 ± 0.29 m^2/g and 9.47 ± 0.52 m^2/g, respectively. Conversely, the other SFPI samples had the lowest EAI compared to their counterparts at other pH levels. DV-SFPI showed an intermediate EAI of 5.65 ± 2.02 m^2/g, followed by DES-SFPI (4.21 ± 1.00 m^2/g), and MV-SFPI (3.72 ± 0.93 m^2/g). Sunflower albumins, which are primarily isolated by salt-based extraction methods, exhibit lower emulsification activity at neutral pH than other protein fractions (González-Pérez et al., 2005).

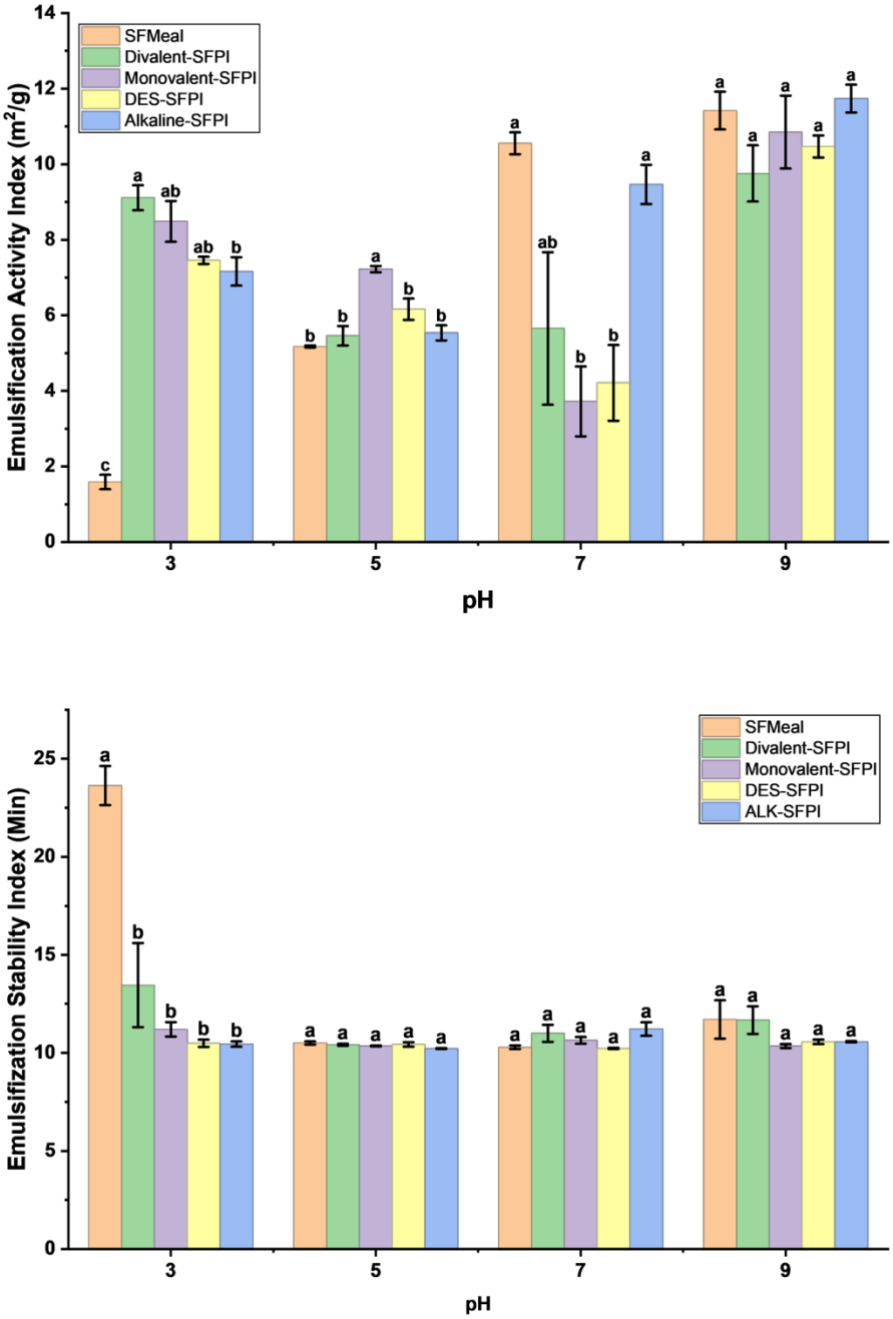

Figure 8. Emulsification activity and stability index for sunflower meal and sunflower protein isolates at different pH conditions.

The high EAI of ALK-SFPI at pH 7 may be due to alkaline extraction-induced structural changes, such as partial unfolding and exposure of hydrophobic groups, which can improve

adsorption at the oil–water interface. The EAI enhanced as pH levels increased, as seen at pH 9 where all samples showed high EAI values with no significant differences among treatments. ALK-SFPI had the highest EAI at 11.74 ± 0.37 m^2/g, followed by SFM at 11.42 ± 0.50 m^2/g, MV-SFPI at 10.85 ± 0.96 m^2/g, DES-SFPI at 10.47 ± 0.29 m^2/g, and DV-SFPI at 9.76 ± 0.74 m^2/g. The increase in EAI at pH 9 is consistent with the greater solubility of SFPI samples at this level. A similar effect was observed by Huang et al. (2024) and Muhammad et al. (2026), where emulsifying properties improved at alkaline pH levels compared to acidic pH near the isoelectric point due to greater protein solubility and surface charge.

The emulsification stability index (ESI) at pH 3 showed SFM with the significantly highest value of 23.63 ± 1.00 minutes, while all SFPI samples were significantly lower, ranging from 10.45–13.46 minutes. As the pH increased, the ESI values had no significant difference among all samples. ESI ranged from 10.22–10.51 minutes at pH 5, 10.23–11.22 at pH 7, and 10.35–11.70 at pH 9. These results indicate that the extraction method had limited influence on emulsion stability at the different pH levels tested, thereby having a limited effect on the ability to create an oil-water interfacial area during homogenization without preventing destabilization after emulsion formation.

EAI values observed in this study were generally higher than those reported by Li et al. (2024), who found EAI values ranging from 3.16 to 4.75 m^2/g for sunflower seed meal proteins from different varieties. Beaubier et al. (2021) also reported that a sunflower protein isolate had a relatively low emulsifying capacity (approximately 25%) but very high emulsion stability (approximately 96%). Overall, the results demonstrate that emulsification activity was strongly affected by both pH and extraction method, whereas emulsion stability was less variable across treatments.

3.4.3.5. Least Gelation Concentration

The classification of soft and hard gels at the minimum required protein concentration are illustrated in Figure 9. Gel formation at a lower protein concentration indicates better gelling ability, while formation of a hard gel indicates stronger network development.

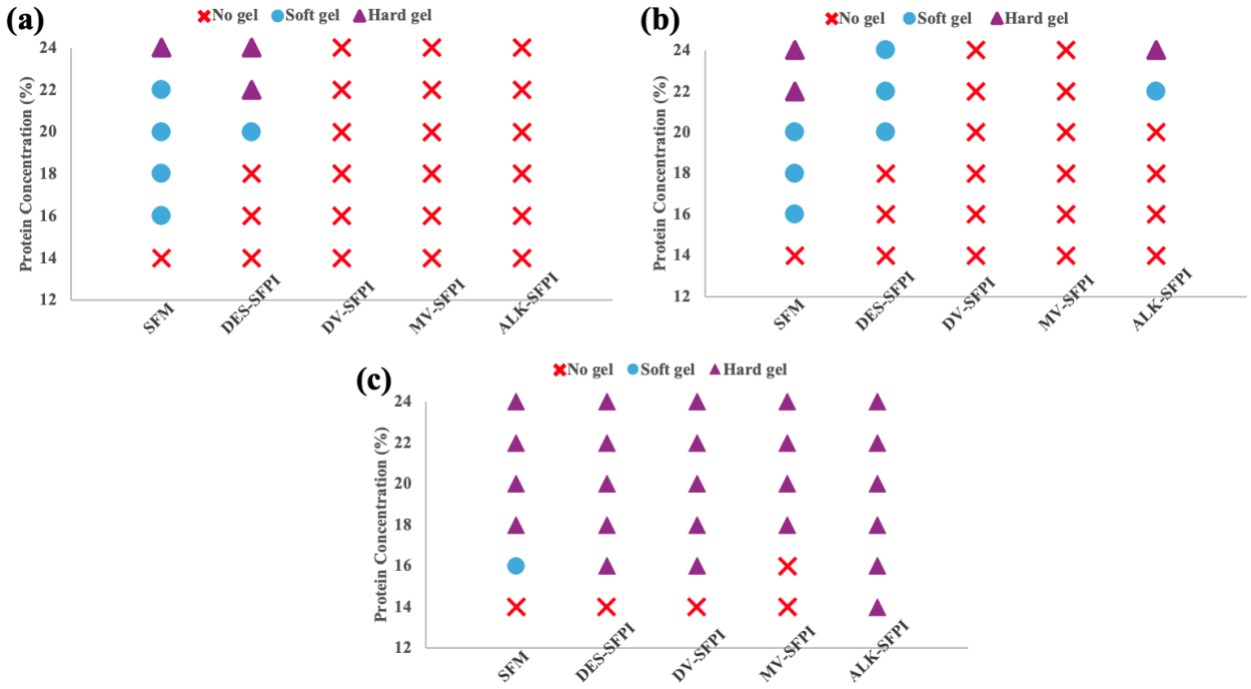

Figure 9. Least gelling capacity for sunflower meal and sunflower protein isolate samples at (a) pH 5, (b) pH 7, (c) pH 9.

At pH 5, only SFM and DES-SFPI formed gels. SFM formed a soft gel at 16% protein concentration and a hard gel at 24%, while DES-SFPI formed a soft gel at 20% and a hard gel at 22%. Sunflower protein fractions, especially 11S globulin helianthinin, are reported to exhibit poor gelation ability and low solubility at acidic and near isoelectric point pH levels (Molina et al., 2004). Therefore, the weak gelation for SFPI samples at pH 5 can be linked to their low protein solubility, thereby lacking the hydration and dispersion required to form gels. However, the ability of SFM to form a soft gel at lower concentrations at pH 5, despite its low solubility, may be due

to the presence of non-protein components in the meal, such as fibre and non-starch carbohydrates. At pH 7, SFM again showed the best gelation among the samples, forming a soft gel at 16% and a hard gel at 22%. DES-SFPI formed only soft gels starting from 20%, while ALK-SFPI formed a soft gel at 22% and a hard gel at 24%. DV-SFPI and MV-SFPI did not gel at any of the tested concentrations. The poor gelation observed at neutral pH may be due to insufficient protein unfolding during heating, which limits exposure of hydrophobic regions and reduces protein-protein interactions necessary for forming a stable gel network (Y. Ma & Chen, 2023; Molina et al., 2004). Furthermore, the presence of polyphenols may reduce gel formation by binding to functional groups on proteins, thereby limiting the availability of reactive sites needed for protein–protein interactions and other matrix components during gel formation (Malik & Saini, 2017; Salgado et al., 2012). The strongest gelation was observed at pH 9, with ALK-SFPI having the best gelation capacity, forming a hard gel at 14%. DES-SFPI and DV-SFPI formed hard gels at 16%, while SFM and MV-SFPI formed hard gels at 18%. This indicates that alkaline conditions, with a higher solubility, greatly improved protein hydration and unfolding, and subsequent protein-protein interactions required for gelation. Molina et al. (2004) also observed that alkaline pH decreased the denaturation temperature of helianthinin and affected its aggregation behaviour. Therefore, sunflower proteins may unfold more readily during heating at 90 °C and pH 9, exposing hydrophobic regions and reactive groups that promote network formation. The gelation results at pH 9 are broadly comparable with reported values for SFPI, but gelation at pH 5 and pH 7 was weaker than many published values. Malik & Saini (2017) reported a minimum gelation concentration of 14% for polyphenol-free SFPI, and 16% for SFPI with polyphenols.

3.5. Conclusion

Overall, the extraction methods significantly influenced the protein purity, extraction yield, structural properties, colour, and techno-functional properties of SFPI obtained from industry-produced SFM. All extraction methods increased protein purity compared to SFM; however, MV-SFPI, DV-SFPI, and DES-SFPI showed significantly higher protein purities of 90.18%, 90.06%, and 88.46%, respectively, compared to ALK-SFPI at 69.35%. DES-SFPI and MV-SFPI also showed the highest protein recovery rates and extraction yields, while ALK-SFPI had the lowest values, indicating that DES and salt-based extractions were more effective in recovering protein from industrial SFM compared to the conventional alkaline-based method.

The extraction methods also induced different structural and physical changes. DV-SFPI showed better preservation of ordered secondary structures, while ALK-SFPI exhibited greater structural rearrangement and aggregation. Colour, an important sensory quality for food applications, was also strongly affected by extraction method, where DV-SFPI was the lightest sample, while ALK-SFPI showed the darkest colour with a green hue.

The techno-functional properties were influenced by both extraction method and pH. MV-SFPI showed the highest solubility at neutral pH, while DES-SFPI had the highest oil holding capacity. Protein isolation improved foaming capacity compared to SFM, with DV-SFPI and DES-SFPI exhibiting strong foaming, particularly under acidic and neutral conditions. Emulsification activity was varied across samples depending on pH and extraction method, whereas emulsion stability showed limited variation. Gelation was strongly pH dependent, with the strongest gelation observed at pH 9. Therefore, DES-based, divalent salt-based, and monovalent salt-based extractions show potential as more sustainable alternatives for producing SFPI with improved or comparable physicochemical and techno-functional properties to conventional alkaline extraction.

Chapter 4: Comparative Analysis of Nutritional Quality and Digestibility of Sunflower Protein Isolates Produced by Sustainable Extraction Technologies

Zainab Husain [ab], Jason Neufeld [a], Deepak Kadam [c], Nandika Bandara [ab]

[a] Department of Food and Human Nutritional Sciences, Faculty of Agricultural and Food Sciences, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada

[b] Richardson Centre for Food Technology and Research, 196 Innovation Drive, Winnipeg, Manitoba R3T 2N2, Canada

[c] JASCO North America, 28600 Mary's Court, Easton, MD 21601, USA

4.1. Abstract

Plant-based protein ingredients must have a high nutritional quality, balanced amino acid composition, and good digestibility to be suitable alternatives to conventional protein sources in food applications. SFM has considerable potential as a protein ingredient due to its favourable nutritional quality. This study evaluated the amino acid composition, IVPD, IV-PDCAAS, anti-nutritional factors, and structural properties of SFPI produced from commercial SFM using three green extraction technologies: divalent salt-based extraction (DV-SFPI), monovalent salt-based extraction (MV-SFPI), and deep eutectic solvent extraction (DES-SFPI). The extracted isolates were compared with the industrial-standard alkaline-extracted SFPI (ALK-SFPI) to determine the influence of extraction method on protein quality. The data were analyzed using ANOVA followed by Tukey's test (n = 3), with statistical significance established at $p < 0.05$. SDS-PAGE confirmed that all SFPI samples retained the characteristic sunflower protein fractions, including 11S helianthinin and 2S albumins. The extraction methods also caused distinct secondary and tertiary

structural changes. MV-SFPI exhibited the most ordered secondary structure, with the highest α-helix content of 54.06% and no detectable unordered structures, whereas DV-SFPI had the highest intramolecular β-sheet and unordered structure contents of 53.24% and 22.46%, respectively. All SFPI samples had broadly comparable amino acid profiles rich in sulfur-containing amino acids, while lysine was identified as the first limiting amino acid. IVPD remained high and significantly similar, except for MV-SFPI, across all samples ranging from 95.36 $\pm$ 0.67% to 98.75 $\pm$ 0.63%. However, the alternative extraction methods produced a significantly higher IV-PDCAAS than ALK-SFPI, with DV-SFPI having the highest score of 62.91 $\pm$ 0.16%. DES-SFPI also had the lowest total phenolic content of 5.30 $\pm$ 0.07 mg GAE/g dry weight, whereas ALK-SFPI had the lowest trypsin inhibitory activity of 0.045 $\pm$ 0.012 TUI/mg dry weight. Overall, these alternative extraction methods exhibit considerable potential for producing SFPI with good nutritional quality and digestibility compared to conventional alkaline-based extraction.

4.2. Introduction

Sunflower meal (SFM) is considered a promising high-quality plant protein source owing to its balanced amino acid composition, high protein content, its potential for food applications, and low levels of antinutritional factors compared to many other plant protein sources (González-Pérez & Vereijken, 2007; Kaur & Ghoshal, 2022). Sunflower proteins primarily consist of two major storage protein fractions: salt-soluble 11S globulins (helianthinin) and water-soluble 2S albumins. These protein fractions influence the techno-functional properties, nutritional quality, and digestibility of sunflower protein isolates (Z. Li et al., 2024). Helianthinin is the predominant protein fraction and consists of acidic and basic polypeptide chains linked by disulfide bonds,

while 2S albumins are smaller proteins that are generally rich in sulfur-containing amino acids (González-Pérez & Vereijken, 2007; Hadidi et al., 2024).

Sunflower proteins are nutritionally valuable, particularly due to the high content of sulfur-containing amino acids methionine and cysteine, which are often limiting in other plant proteins including legumes and pulses (Kaur & Ghoshal, 2022). However, lysine is consistently reported as the first limiting amino acid in sunflower meal and sunflower protein isolates, which reduces the amino acid score and can limit the overall protein quality (Alexandrino et al., 2017; Hadidi et al., 2024). This makes sunflower protein a useful complementary plant protein source when combined with lysine-rich proteins. Therefore, evaluating the amino acid composition and amino acid score of sunflower protein isolates is essential to determine the effect of different extraction methods on indispensable amino acids and the nutritional value of the protein isolate ingredient.

Protein digestibility is another major determinant of protein nutritional quality. Sunflower proteins generally exhibit good digestibility, with previous studies reporting high in vitro digestibility values for sunflower protein isolates and concentrates produced through alkaline extraction (Alexandrino et al., 2017; Salgado et al., 2012). Various factors affect the digestion properties of sunflower protein, such as polyphenol content which interact with the protein using non-covalent bonds and stimulate protein hydrolysis that favours protein digestion, as well as the protein secondary structures where the preserved high content of ordered alpha-helix and beta-sheet content can have higher digestibility (X. Huang et al., 2024). Furthermore, the structure of sunflower proteins is closely related to their digestibility and nutritional quality due to the protein conformation influencing the accessibility of peptide bonds to digestive enzymes (Carbonaro et al., 2012; Tang et al., 2022).

A major limitation to the use of sunflower meal as a protein source is the presence of phenolic compounds, particularly chlorogenic acid (CGA), which comprises approximately 70–75% of the total phenolic composition (Alagawany et al., 2015; Wildermuth et al., 2016). CGA, considered an anti-nutrient, can lead to phenolic-protein interactions that inhibit the access of proteolytic digestive enzymes, leading to a reduction in the digestibility of SFPI (Alagawany et al., 2015; Bau et al., 1983; González-Pérez & Vereijken, 2007). In opposition to this, some studies found that while CGA in sunflower protein can slightly reduce digestibility, it remained high enough and was not considered limiting for human and animal feeding (Salgado et al., 2012; Treviño et al., 1998). Mainly, CGA can restrict the potential of SFM as a protein source due to the green or brown colour factors that it imparts on sunflower protein concentrates and isolates at alkaline pH (Alagawany et al., 2015). The removal of CGA from sunflower meal has been investigated using pre-extraction methods utilizing aqueous organic solvents (such as methanol and ethanol), salts (such as sodium bisulfite), reducing agents, membrane filtration, and washing methods using isopropanol after protein isolation (Alexandrino et al., 2017; Bau et al., 1983; Wildermuth et al., 2016). However, these processes are not suitable for industrial scale-up and raise sustainability concerns related to chemical disposal.

In addition, trypsin inhibitors are considered anti-nutritional factors as they inhibit digestive proteases such as trypsin and chymotrypsin, which can reduce protein hydrolysis and amino acid availability (Avilés-Gaxiola et al., 2018; Samtiya et al., 2020). Trypsin inhibitor content in sunflower is low compared to other plant protein sources. Some sunflower seed varieties are relatively free from trypsin inhibitors (Roy & Bhat, 1974). Nonetheless, extraction conditions can play a major role in reducing trypsin inhibitory activity (TIA) through protein denaturation or the removal of inhibitor fractions in the soluble phase during extraction.

Conventional alkaline-based extraction is widely used for producing plant protein isolates, especially for industrial applications. However, the harsh extraction conditions of this method can form protein-phenolic complexes, promote protein denaturation and CGA oxidation, and induce amino acid modifications through lysine and oxidized CGA interactions (Alexandrino et al., 2017; Ishii et al., 2021). For plant proteins to be suitable as alternative protein ingredients, they must provide good nutritional quality while maintaining low levels of antinutritional factors. Protein quality is determined by the total protein content, the balance of indispensable amino acids, the extent of protein digestibility, and bioavailability of amino acids (Hadidi et al., 2024; Sarwar Gilani et al., 2012).

It is hypothesized that the extraction methods would significantly affect the nutritional quality and structural characteristics of SFPI, and that the green extraction technologies would produce SFPI with an improved amino acid profile, digestibility, and IV-PDCAAS, with a preserved native protein structure and acceptable levels of anti-nutritional factors relative to conventional alkaline extraction. The objective of this study was to determine how the green extraction technologies (divalent salt-based, monovalent salt-based, and DES methods) influence the amino acid profile, digestibility, anti-nutritional profile, and the secondary and tertiary structural characteristics of SFPI produced from commercial SFM. SFPI obtained using the alternative extraction methods were compared with conventionally produced alkaline-extracted SFPI to determine the feasibility of replacing it on an industrial scale.

4.1. Materials and Methods

4.1.1. Raw Materials and Chemicals

The SFM pellets, a by-product of oil extraction at 60 °C, was obtained from Richardson Centre for Food Technology and Research milling facility (Winnipeg, MB, Canada). The Folin–Ciocalteu reagent and gallic acid were purchased from Fisher Scientific (Ottawa, ON, Canada). Trypsin from porcine pancreas (lyophilized powder, Type IX-S, 000–20,000 BAEE units/mg protein), ∝-chymotrypsin from bovine pancreas (lyophilized powder, Type II, ≥ 40 units/mg protein), protease from Streptomyces griseus (Type XIV, ≥ 3.5 units/mg), and benzoyl-DL-arginine-4-nitroanilide were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals used were analytical grade.

4.1.2. SFPI Sample Preparation

The milled SFM pellets (produced as described in Section 3.3.2) were used to extract protein isolates using the conventional alkaline-based extraction (Section 3.3.3), monovalent and divalent salt-based extractions (Section 3.3.4), and DES extraction (Section 3.3.5). SFPI samples were stored at 4 °C until further analysis.

4.1.3. Structural Characterization of SFPI

4.1.3.1. Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS-PAGE) Analysis

Identification of protein composition of SFM flour and SFPI based on molecular weight migration was analyzed using the SDS-PAGE method as described by Hewage et al. (2024). Protein samples were dispersed in 5% sodium dodecyl sulfate solution and heated at 85 °C for 1 hour, then centrifuged at 12,000 ×g for 10 minutes. The supernatant was mixed with an equal

volume of Laemmli buffer to obtain a protein concentration of 2 mg/mL, and 2-mercaptoethanol was added with the Laemmli buffer for reducing conditions. The samples were then denatured at 90 °C for 5 minutes and loaded (6 μL) onto a 4-15% Mini-Protean® TGX^{TM} precast. 10 μl of protein molecular weight standard (Precision Plus ProteinTM, Bio-Rad Laboratories Inc., CA, USA) was run alongside the samples. Electrophoresis was carried out in a running buffer at a constant voltage of 150 V for 1 hour in a Mini-Protein electrophoresis unit (Bio-Rad laboratories Inc, CA, USA). Following electrophoresis, the gel was stained using Coomassie Brilliant Blue R-250 staining solution for 1 hour, then destained with 50% methanol and 10% acetic acid solution for 2 hours. The protein bands were imaged using the Bio-Rad ChemiDocTM Imaging system (Bio-Rad Laboratories Inc, CA, USA).

4.1.3.2. Secondary Structure Analysis

The secondary structure analysis of extracted SFPI samples was conducted as described in Section 3.3.7.1 using FTIR (Invenio FTIR spectrometer, Bruker, MA, USA) at a 400–4000 cm^{-1} wavenumber range and 4 cm^{-1} resolution using 120 scans. Origin 2024 software (Origin lab cooperation, MA, USA) was used to further analyze the FTIR spectra using the 2nd derivative, smoothed with the Savitzky-Golay function, and amide I peaks (at 1600–1700 cm^{-1} range) were fitted for identification of corresponding secondary structure components and their proportions (Tintor et al., 2024).

4.1.3.3. Circular Dichroism Analysis

Near-UV circular dichroism spectroscopy was used to analyze the tertiary structure of SFPI samples following the method of Ajibola et al. (2017) with minor changes. The spectra were measured on a JASCO J-815 CD Spectrometer (JASCO, Tokyo, Japan). SFPI samples were solubilized at a 2 mg/mL concentration in 0.1 M phosphate buffer (pH 7), then centrifuged at

10,000 ×g for 30 minutes and the supernatant was collected for analysis. Near-UV spectra were measured at 250-350 nm at 25 °C using a quartz cuvette with a 1 mm path length. Spectra were obtained as the average of three consecutive scans and the corresponding buffer spectra were automatically subtracted.

4.1.4. SFPI Quality Characterization

4.1.4.1. Anti-Nutritional Factors

Total phenolic content (TPC) was determined following the method of Ainsworth & Gillespie (2007) with changes. Samples were prepared by dispersing 0.1 g of SFPI in 1 mL of 40% acetone solution and TPC was extracted by stirring in a water bath at 40 °C at 200 rpm for 1 hour. The extract was centrifuged at 4,000 rpm for 20 minutes and the supernatant was obtained for further extraction following the same procedure. The final supernatant was obtained for TPC analysis. Sample extract (20 μL) was mixed with 1.58 mL distilled water and 100 μL folin-ciocalteu reagent and incubated for 8 minutes. Immediately after, 300 μL of 1.88 M Na_2CO_3 solution was added and incubated for 2 hours. Absorbance measurements were read at 765 nm against a gallic acid standard curve.

Trypsin inhibitory activity (TIA) was determined according to the method of Kim & Kirshnan (2023) and American Oil Chemists' Society (AOCS) (2017) with minor changes. SFPI samples (0.5 g) were extracted in 25 mL 0.01 M NaOH for 1 hour at 300 rpm at room temperature. The extract was then centrifuged at 12,000 rpm for 10 minutes, and 2 mL of the supernatant was obtained and diluted in 8 mL distilled water (1:5). The extracted samples (1 mL) were mixed with 2 mL of 185 mg/L trypsin solution and 5 mL benzoyl-DL-arginine-4-nitroanilide in dimethyl sulfoxide (BAPNA-DMSO) solution and incubated for 10 minutes. The reaction was immediately

terminated by adding 1 mL of 30% acetic acid and the absorbance was measured at 410 nm. The TIA is expressed as trypsin inhibitor units (TUI) per mg of dry sample using Equation (11).

$$TUI\ per\ mL = \frac{\left[\left(A_{410\ Ref} - A_{410\ Ref\ blank}\right) - \left(A_{410\ Sample} - A_{410\ Sample\ blank}\right)\right]}{0.01 \times mL\ of\ extracted\ sample} \quad (10)$$

Using Equation (10) in Equation (11):

$$TUI\ per\ mg\ dry\ sample = \frac{TUI\ per\ mL}{mg\ of\ dry\ sample\ used\ for\ extraction} \quad (11)$$

4.1.4.2. Amino Acid Composition and Amino Acid Score

The total amino acid content was analyzed using standardized wet chemistry methods with slight modifications. Regular amino acids concentration was analyzed by acid hydrolysis (AOAC 982.30 method), sulfur-containing amino acids (methionine and cysteine) were analyzed by performic acid oxidation (AOAC 994.12 method) (AOAC International, 2012), and tryptophan was analyzed by alkaline hydrolysis (ISO 13904:2005) (International Organization for Standardization (ISO), 2005). Regular and sulfur-containing amino acids were derivatized using Water AccQ-Tag reagent and run on Nexara 2 Ultra-performance liquid chromatography (UPLC) (Shimadzu, Kyoto, Japan) equipped with a 10 cm AccQ-tag Ultra C18 1.7 μm column for regular and sulfur-containing amino acids, and a 15 cm Phenomenex Luna C18 3.0 μm column for tryptophan.

The amino acid score (AAS) was calculated to evaluate protein quality based on essential amino acid composition in SFPI samples using preschool children (2-5 years old) scoring as a reference pattern (Food and Agriculture Organization of the United Nations (FAO) & World Health Organization (WHO), 1991). The amino acid score was calculated individually for all essential amino acids using Equation (7), and the essential amino acid that yielded the lowest AAS value

was identified as the first limiting amino acid, and this value was taken as the overall AAS of the protein.

$$AAS\ (\%) = \frac{mg\ of\ amino\ acid\ in\ 1\ g\ of\ test\ protein}{mg\ of\ amino\ acid\ in\ 1\ g\ of\ reference\ pattern\ protein} \times 100 \qquad (7)$$

4.1.4.3. In Vitro Protein Digestibility and In Vitro Protein Digestibility Corrected Amino Acid Score

In vitro protein digestibility (IVPD) of SFPI samples was determined using the pH-drop method (Hsu et al., 1977; Tinus et al., 2012) with slight modifications. A multi-enzyme solution was prepared with 1.6 mg/mL trypsin from porcine pancreas (lyophilized powder, Type IX-S, 000–20,000 BAEE units/mg protein), 3.1 mg/mL ∝-chymotrypsin from bovine pancreas (lyophilized powder, Type II, ≥ 40 units/mg protein), and 1.3 mg/mL protease from Streptomyces griseus (Type XIV, ≥ 3.5 units/mg). The multi-enzyme solution was stirred at 37 °C at 350 rpm until solubilized, the initial pH was recorded then adjusted to 8.00 ∓ 0.05 with 0.1 M HCl or NaOH. SFPI samples were weighed to contain 10.0 ∓ 0.5 mg nitrogen (1 mg N/mL) using Equation (8) and dispersed in 10 mL HPLC grade water.

$$Sample\ weight\ (mg) = \frac{10\ mg}{Nitrogen\ factor\ \%} \qquad (8)$$

The sample solution was stirred at 37 °C at 450 rpm until dissolved, then the pH was adjusted to 8.00 ∓ 0.05 and recorded. 1 mL of the multi-enzyme solution was then immediately added. After 10 minutes, the final pH of the sample solution was recorded. The IVPD was calculated using Equation (9).

$$IVPD = 76.97 + (19.16 \times \Delta pH_{10min}) \qquad (9)$$

The in vitro protein digestibility corrected amino acid score (IV-PDCAAS) was calculated as the product of IVPD and the lowest AAS, corresponding to the first limiting essential amino acid in each sample, using the preschool children (2–5 years) reference pattern.

4.1.5. Statistical Analysis

Statistical analysis of data was conducted using OriginPro software (version 2024, OriginLab Corporation, MA, USA). All the data was analyzed using Analysis of Variance (ANOVA) with Tukey's test for means comparison at a significance level of $P < 0.05$. All analyses were carried out in triplicates.

4.2. Results and Discussion

4.2.1. Structural Characterization of SFPI

4.2.1.1. SDS-PAGE Analysis

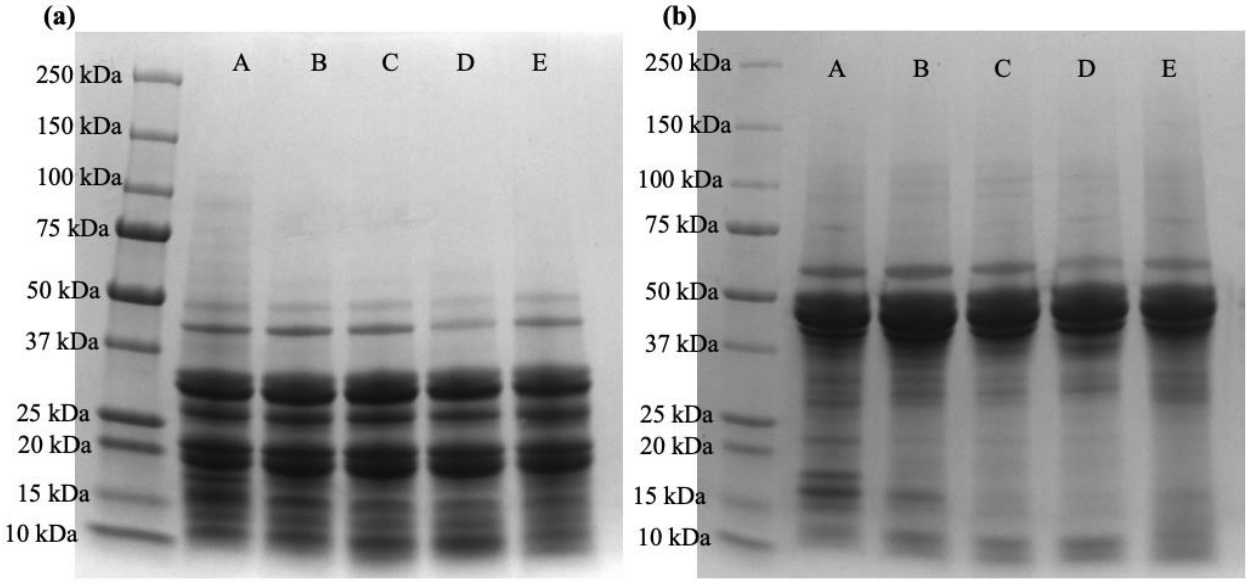

Figure 10. SDS-PAGE (a) reducing and (b) non-reducing profiles of sunflower meal flour and extracted protein isolates. Lane A – Sunflower meal flour (SFM), Lane B – Deep eutectic solvent extracted sunflower protein isolates (DES-SFPI), Lane C – Divalent salt extracted sunflower

protein isolates (DV-SFPI), Lane D – Monovalent salt extracted sunflower protein isolates (MV-SFPI), Lane E – Alkaline extracted sunflower protein isolates (ALK-SFPI).

The major sunflower protein fractions are illustrated in the SDS-PAGE reducing and non-reducing profiles in Figure *10*. Similar characteristic protein bands were observed in both the SFM and SFPI samples in varying intensity likely indicating difference in concentration. Helianthinin, the predominant sunflower protein fraction, is comprised of six polypeptide subunits assembled into an oligomeric 11S globulin complex with a molecular weight ranging from 300–350 kDa (Kaur & Ghoshal, 2022). The subunits contain acidic (32–44 kDa) and basic (21–27 kDa) polypeptide chains linked together with disulfide bonds. The acidic polypeptide monomers were observed in the SDS-PAGE reducing profile at ~32 kDa and ~44 kDa with similar intensities across all samples, although the MV-SFPI and ALK-SFPI samples exhibited a less pronounced band intensity at ~44 kDa. The basic polypeptide chain monomers were identified at ~27 and ~21 kDa. Moreover, the second major 2S sunflower albumins mainly consist of single polypeptide chains ranging from 10-18 kDa (Hadidi et al., 2024). In the reducing profile, distinct bands were observed at ~10–20 kDa across all SFM and SFPI samples corresponding to the molecular weight of sunflower albumins. Bands at ~12 and ~16 kDa are consistent with the 2S methionine-rich albumin and 2S storage albumin protein fractions, respectively. Band intensities of 2S albumins varied across extraction methods, with the MV-SFPI and ALK-SFPI samples having less pronounced bands than DES-SFPI and DV-SFPI suggesting lower extraction of these protein fractions.

The SDS-PAGE reducing conditions produced clearer separated protein bands due to the disruption of the disulfide bridges. On the other hand, the dense band region at ~37–50 kDa in the non-reducing profile may represent intact oligomeric 11S globulin polypeptide subunits with disulfide bonds that remained associated. Helianthinin bands can also be observed at ~60 kDa in

the non-reducing profile, with the MV-SFPI and ALK-SFPI samples having the lowest intensity potentially linked to the protein denaturation or aggregation of 11S globulins acidic polypeptide bands under these extraction conditions (Ishii et al., 2021). Furthermore, the faint bands observed above ~75 kDa may indicate the presence of protein aggregates formed through disulfide crosslinking or protein-polyphenol interactions induced by the extraction methods (Thapa et al., 2025).

4.2.1.2. Secondary Structure Analysis

The FTIR deconvolution of the amide I region are illustrated in Figure *11*. This shows that the extraction method strongly influenced the protein secondary structure. The secondary structure of sunflower meal protein is dominated by β-sheets (Z. Li et al., 2024). This is evident in DES-SFPI, DV-SFPI, and ALK-SFPI samples with values ranging from 46.34–53.24%. In contrast, MV-SFPI had the lowest β-sheet content (32.55%) between samples, but the highest α-helix content of 54.06%. Across all four samples, a higher α-helix and intramolecular β-sheet content reflects a more ordered protein secondary structure. However, detecting β-sheet aggregation or unordered structures may indicate partial unfolding resulting from the extraction method or various interactions.

Looking at the secondary structure relative content in Table 4-1, DV-SFPI had the highest intramolecular β-sheet content (53.24%), detectable β-sheet aggregates (3.17%) and β-turns (8.22%), and the lowest α-helix content (12.46%). DES-SFPI contained 35.13% α-helix, 46.34% intramolecular β-sheets, and 7.51% β-turns. MV-SFPI had the highest α-helix content of 54.06%, detectable β-sheet aggregates (3.36%), and the lowest intramolecular β-sheet content of 32.55%. Lastly, ALK-SFPI showed high intramolecular β-sheet content (53.19%), comparable α-helix content (26.78%) and β-turns (9.85%). Both the DES-SFPI and ALK-SFPI samples had no

detectable β-sheet aggregates. DV-SFPI showed the highest unordered structure content (22.46%) and a small amount of β-sheet aggregation (3.17%), likely due to the disruption of the native hydrogen bonds through phenolic-protein non-covalent interactions (Karefyllakis et al., 2017; Malik et al., 2016). On the other hand, the DES-SFPI and ALK-SFPI samples had a lower content of unordered structure of 11.02% and 10.09%, respectively. This indicates that the DES and alkaline-based extraction methods induced partial structural disorder, while preserving majority of the protein secondary confirmation usually observed in sunflower meal protein.

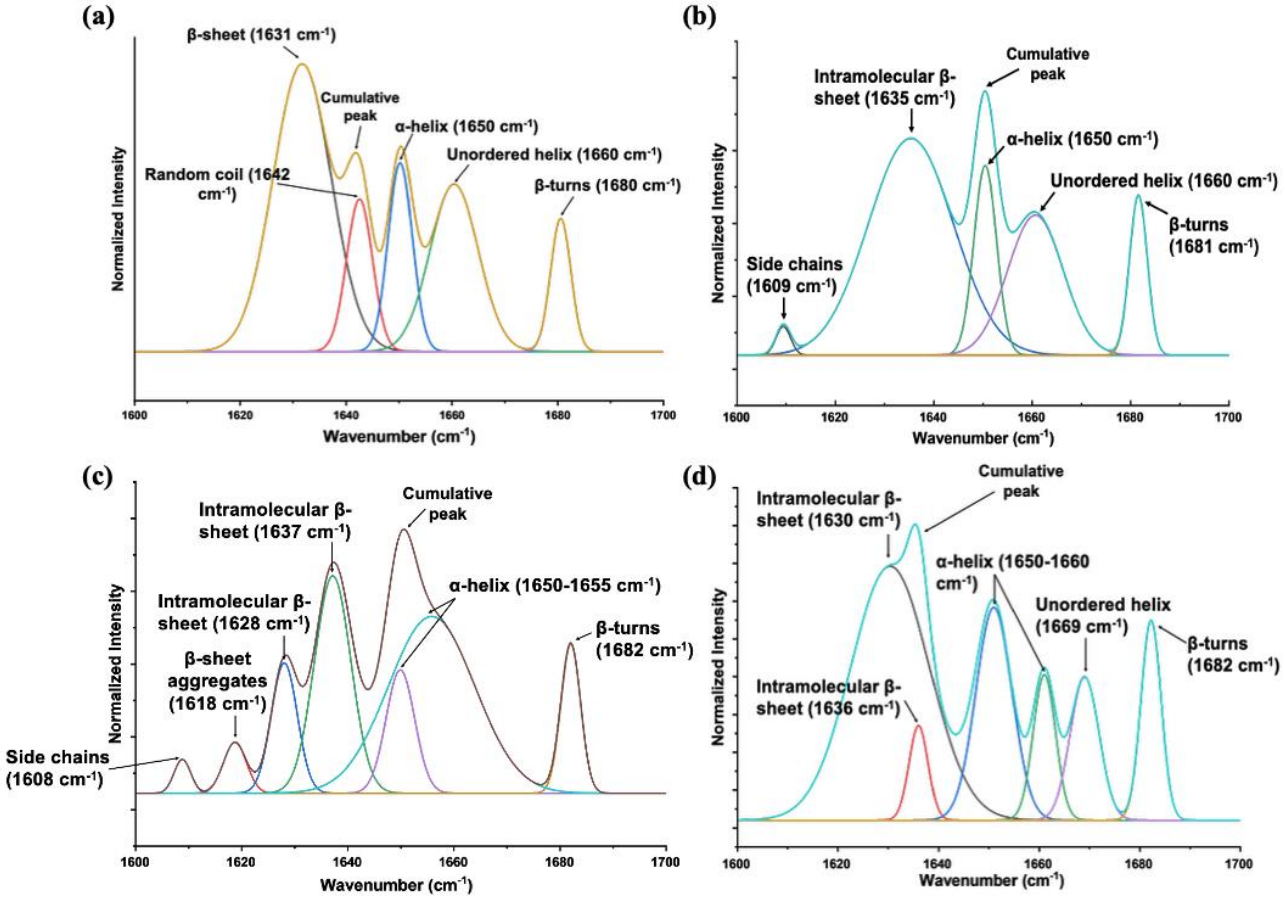

Figure 11. Protein secondary structural changes in extracted sunflower protein isolate (SFPI) samples. (a) Deep eutectic solvent extracted sunflower protein isolates (DES-SFPI), (b) Divalent

salt extracted sunflower protein isolates (DV-SFPI), (c) Monovalent salt extracted sunflower protein isolates (MV-SFPI), (d) Alkaline extracted sunflower protein isolates (ALK-SFPI).

Moreover, MV-SFPI was the only sample with no unordered structure detected and a small amount of β-sheet aggregation (3.36%), suggesting that this extraction better preserved ordered secondary structure and limited unfolding compared to the other extraction methods. Changes to the sunflower protein secondary structure can be related to the in-vitro digestibility and nutritional quality of the SFPI samples. Carbonaro et al. (2012) reported reduced digestibility for plant proteins with a high content of β-sheets due to its hydrophobic nature and hydrophobic interactions that promote aggregation, which limits the accessibility of protein sites for digestive enzymes. This effect may be present in the DV-SFPI and MV-SFPI samples, with β-sheet aggregates detected, where protein-protein interactions can decrease digestibility. Furthermore, the significant increase of chlorogenic acid phenolic-protein interactions (with 22.46% relative content of unordered structures) found in the DV-SFPI sample can also lead to decreasing amino acid bioavailability and digestive enzymes binding inhibition. In contrast, the unordered structures in DES-SFPI and ALK-SFPI samples may indicate protein unfolding, which can improve the accessibility of peptide bonds to digestive enzymes (Tang et al., 2022).

Table 4-1. Relative proportions of protein secondary structures extracted sunflower protein isolate (SFPI) samples.

Sample	Secondary structure relative content (%)					
	Side chains	α-helix	β-turns	Intramolecular β-sheets	β-sheet aggregates	Unordered structures
DES-SFPI	-	35.13	7.51	46.34	-	11.02
DV-SFPI	1.20	12.46	8.22	53.24	3.17	22.46
MV-SFPI	1.61	54.06	8.41	32.55	3.36	-
ALK-SFPI	-	26.78	9.85	53.19	-	10.09

4.2.1.3. Circular Dichroism Analysis

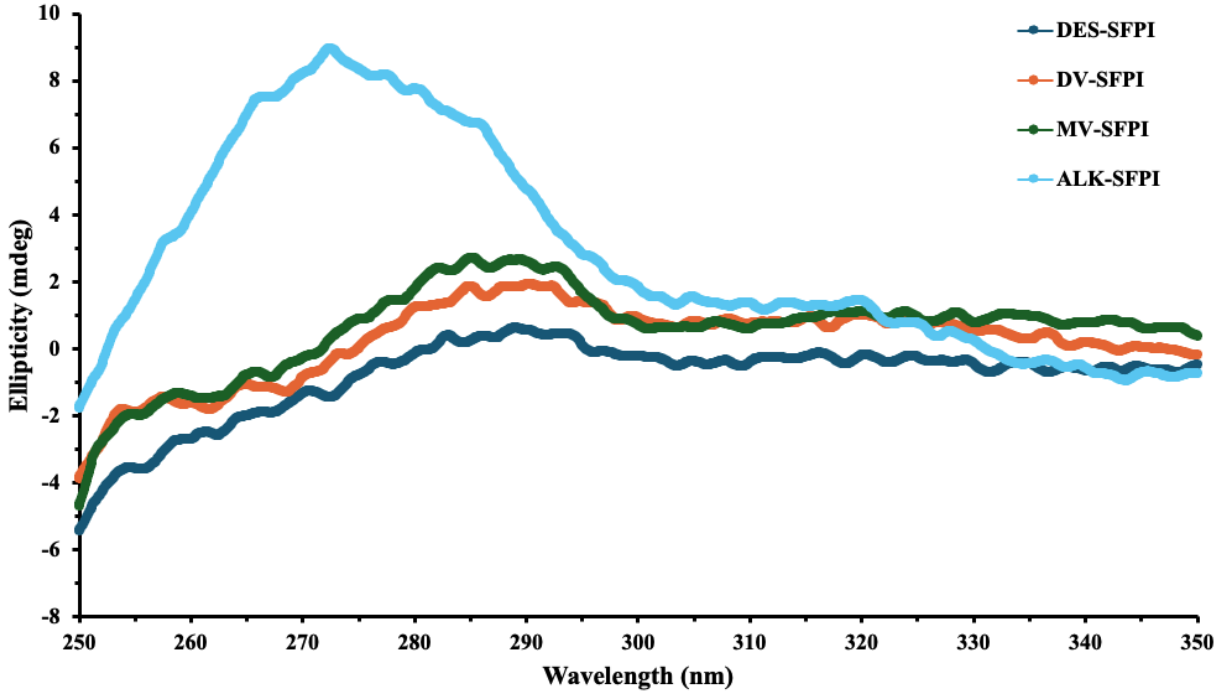

Figure 12. Near-UV circular dichroism (CD) spectra illustrating tertiary structural changes in sunflower protein isolates (SFPI) samples.

The near-UV region (250–350 nm) is a fingerprint of aromatic amino acids and disulfide bonds, giving insight on the tertiary structural changes of sunflower protein isolates obtained using the four extraction methods. The near-UV spectra of the SFPI samples (Figure *12*) showed varying positive ellipticity within 260–300 nm, which is consistent with literature reports for helianthinin, the major 11S globulin protein fraction in sunflower. Helianthinin has been reported to exhibit positive near-UV CD bands in the same range, with a maximum peak at 285 nm (González-Pérez & Vereijken, 2007). Moreover, González-Pérez & Vereijken (2007) stated that sunflower helianthinin resembles other 11S globulin storage proteins such as soy glycinin and rapeseed 11S globulin in its structural behaviour. The spectral differences observed within the 250–300 nm region can be related to changes in aromatic amino acid environments. Signals around 250–270 nm, 270–280 nm, and 280–300 nm are commonly associated with phenylalanine, tyrosine, and tryptophan, respectively (Kelly et al., 2005; Rivera-Najera et al., 2014).

Differences in the CD positive signal intensity within the 260–300 nm region indicate that the extraction methods influenced the tertiary structure conformation of sunflower proteins. The ALK-SFPI sample exhibited the highest positive ellipticity between 260 and 290 nm, with a maximum peak at approximately 273 nm. The higher intensity and shift in peak position suggest rearrangement or exposure of aromatic residues (phenylalanine and tyrosine), which may reflect tertiary structure disruptions under alkaline conditions. Similar processing conformational changes were observed in soy 11S globulin (glycinin) which had disulfide bond disruptions and tertiary structure rearrangement under processing conditions such as temperature, pH, and ionic strength (Lakemond et al., 2000). The MV-SFPI and DV-SFPI samples showed similar moderate positive ellipticity around 275–295 nm, suggesting less pronounced tertiary structural changes involving tyrosine and tryptophan environments compared to ALK-SFPI. Compared to the other samples,

DES-SFPI displayed the lowest ellipticity across the near-UV region with a weak positive ellipticity around 280–295 nm. This may show a partial tertiary structural disruptions and less defined aromatic amino acid environments (Chukwuejim & Aluko, 2026).

4.2.2. Sunflower Protein Isolate Quality Characterization

4.2.2.1. Anti-Nutritional Factors

The results presented in Table *4-2* show no significant difference in TPC across DV-SFPI, MV-SFPI, and ALK-SFPI which had higher total phenolic content (TPC) ranging from 8.66–9.54 mg GAE/g DW. In contrast, DES-SFPI had a significantly lower TPC of 5.30 ± 0.07 mg GAE/g DW compared to the other SFPI samples. A study evaluating DES extraction on lupin protein isolates has similarly seen a significant reduction in TPC compared to alkaline extraction (Nadeeshani et al., 2026). Furthermore, the total phenolic content obtained in this study was within the lower range of 7.51–18.51 mg GAE/g DW reported for SFM by Zardo et al. (2019), and 9.82 ± 0.02 mg GAE/g DW reported by Hallouch et al. (2025). A higher phenolic content can promote phenolic-protein interactions, disrupting ordered protein secondary structures and increasing the proportion of unordered conformations (Malik et al., 2016). This is supported by the DV-SFPI sample, which exhibited the highest phenolic content (9.54 mg GAE/g DW) and the highest unordered secondary structures content (22.46%). Phenolic compounds can also inhibit digestive enzymes, thus reducing the protein digestibility. However, the results in this study do not show a conclusive effect of phenolic content on protein digestibility. Similarly, an animal study evaluating the anti-nutritional effect of chlorogenic acid on broiler chicks found that 6 g of CGA does not significantly affect the protein digestibility (Treviño et al., 1998). In addition, Salgado et al. (2012) reported a high digestibility of 95.40% despite samples containing 2.5 g/100 g phenolic compounds.

Table 4-2. Total phenolic content (TPC) and trypsin inhibitory activity (TIA) for sunflower protein isolates obtained using four extraction methods.

	TPC (mg GAE/g DW)	TIA (TUI/mg DW)
DES-SFPI	5.30 ± 0.07^{B}	0.167 ± 0.001^{B}
DV-SFPI	9.54 ± 0.30^{A}	0.239 ± 0.003^{A}
MV-SFPI	8.66 ± 0.28^{A}	0.226 ± 0.006^{A}
ALK-SFPI	9.00 ± 0.36^{A}	0.045 ± 0.012^{C}

$^{A-C}$ Superscripts denote a significant difference between samples, $P < 0.05$ using Tukey's test.

There is no significant difference in TIA across DV-SFPI and MV-SFPI, which had the highest TIA values of 0.239 ± 0.003 and 0.226 ± 0.006 TUI/mg DW, respectively. Conversely, DES-SFPI had a significantly lower TIA of 0.167 ± 0.001 TUI/mg DW, while ALK-SFPI exhibited the lowest TIA of 0.045 ± 0.012 TUI/mg DW compared to the other SFPI samples. The four extraction methods significantly affected TIA. This is consistent with Wintersohle et al. (2023), who reported that different extraction procedures significantly influenced TIA in mung bean protein isolates. The lower TIA observed in ALK-SFPI suggests that alkaline extraction may be more effective in reducing trypsin inhibitory activity, possibly due to protein denaturation or the solubilisation of trypsin inhibitor fractions in the supernatant after protein precipitation (Baker & Rackis, 1986; Frota et al., 2018). This effect was also observed by Frota et al. (2018), where alkaline isoelectric precipitation decreased TIA from 32.5 ± 0.5 TUI/mg protein in cowpea flour to 8.3 ± 0.2 TUI/mg protein in cowpea protein isolates. On the other hand, the higher TIA in DV-SFPI and MV-SFPI may indicate greater retention or co-extraction of trypsin inhibitor fractions under these extraction conditions. Nonetheless, the TIA values observed in this study were relatively low, and therefore TIA may not majorly affect protein digestibility. Studies reporting

TIA in sunflower protein isolates are extremely limited. Salgado et al. (2011) reported negligible TIA in sunflower meal used for protein concentrate and isolate preparation. On the other hand, a study conducted by Colgrave et al. (2010) used sunflower seeds as a significant source to isolate trypsin inhibitor-1 (SFTI-1) for its potent trypsin inhibitory activity to be used in a peptide-based drug template.

4.2.2.2. Amino Acid Composition and Amino Acid Score

The SFPI samples exhibited a broadly similar amino acid profile, as shown in Table 4-*3*, with slight differences in amino acid composition across the four extraction methods. Although the ALK-SFPI sample has higher amino acid concentrations when expressed on a protein basis, this likely reflects normalization against its lower protein purity (69.35%) and should be considered when interpreting its overall nutritional value and the AA content per unit of protein isolate. The total amino acid content (g/100 g protein) was relatively comparable in all four samples, with ALK-SFPI exhibiting the highest total amino acid content of 136.65 g/100 g protein, followed by DV-SFPI (110.99 g/100 g protein), and DES-SFPI and MV-SFPI had similar total amino acid content of 107.70 and 107.64 g/100 g protein, respectively.

The amino acid composition of the SFPI samples is characterized by a high glutamic acid (Glu) content (261.64–322.69 mg/g protein), followed by aspartic acid (Asp) (104.75–147.10 mg/g protein) and arginine (Arg) (99.09–129.62 mg/g protein), which is consistent with the composition reported for various sunflower meal varieties in literature (Z. Li et al., 2024). The glutamic acid, aspartic acid, and arginine contents found in this study are also comparable to the values reported for protein isolates produced from Bulgarian sunflower meal (Ivanova et al., 2013). Moreover, most essential amino acids in the four SFPI samples met or exceeded the ideal protein pattern. Isoleucine AAS ranged from 166.79% to 218.25%, aromatic amino acids (Phe + Tyr) from

123.92% to 177.82%, and leucine from 99.54% to 130.14%. This demonstrates that the SFPI samples were particularly rich in branched-chain and aromatic amino acids, consistent with previous findings for sunflower meal and protein ingredients (Oseyko et al., 2020; Villamide & San Juan, 1998). The SFPI samples also contained relatively high histidine (His) contents which exceeded the ideal protein pattern (135.97%–185.16%) with similar values as reported by Zaky et al. (2022). Histidine (His) is nutritionally relevant as an indispensable amino acid requirement pattern for children and the elderly, while arginine (Arg) is valuable for metabolic and immune-related physiological functions (Pillai & Kurpad, 2012; Wu et al., 2009). The amino acid profiles of the SFPI samples showed a clear abundance of both hydrophobic and hydrophilic amino acids. The samples contained high levels of hydrophobic amino acids such as leucine, isoleucine, valine, and tryptophan. Oseyko et al. (2020) similarly reported that the processing of sunflower flour resulted in enrichment in hydrophobic amino acids and had a higher hydrophobic/hydrophilic amino acid ratio.

Lysine (Lys) was identified as the first limiting amino acid based on the AAS values of 60.43–73.53%, with concentrations of 35.05–42.65 mg/g protein, which are slightly higher than values reported by Ivanova et al. (2013). Numerous studies have also reported lysine as the first limiting amino acid in SFM and SFPI (Alagawany et al., 2015; Alexandrino et al., 2017; Hadidi et al., 2024; Saleh et al., 2021). The low lysine content in SFPI may be attributed to the interaction of lysine with sunflower meal components during oil extraction forming lysine complexes, thus reducing its extractability compared to the other amino acids (Ivanova et al., 2013). Despite the low lysine content, all SFPI samples contain a high content of sulfur-containing amino acids (methionine and cystine) ranging from 47.38–55.48 mg/g protein. ALK-SFPI had the highest sulfur amino acid score of 221.93%, followed by DV-SFPI (211.69%) and MV-SFPI (210.09%).

The AAS range for sulfur-containing amino acids (Met + Cys) found in this study is consistent with values reported in literature varying between 99.14–258% (Ivanova et al., 2013; Oseyko et al., 2020). In contrast, the DES-SFPI sample had the lowest sulfur amino acid score (189.54%) amongst the four samples, but it still exceeds the expected ideal protein pattern. The high sulfur-containing amino acid content positions sunflower protein as a valuable complementary plant protein source compared to other plant sources such as legumes and pulses with lower limiting sulfur amino acid content (Grela et al., 2017; Iqbal et al., 2006).

Table 4-3. Indispensable amino acid (IAA), dispensable amino acid (DAA) composition, and the amino acid score (AAS) percentages for sunflower protein isolates obtained using four extraction methods.

AA composition	DES-SFPI		Divalent-SFPI		Monovalent-SFPI		Alkaline-SFPI	
	AA (mg/g protein)	AAS (%)	AA (mg/g protein)	AAS (%)	AA (mg/g protein)	AAS (%)	AA (mg/g protein)	AAS (%)
Indispensable (IAA)								
Histidine (His)	26.74	140.72	26.50	139.49	25.83	135.97	35.18	185.16
Isoleucine (Ile)	47.19	168.53	47.22	168.66	46.70	166.79	61.11	218.25
Leucine (Leu)	66.76	101.16	67.35	102.05	65.70	99.54	85.89	130.14
Lysine (Lys)	35.05	60.43	40.97	70.63	40.03	69.03	42.65	73.53
Threonine (Thr)	35.13	103.32	37.69	110.85	35.96	105.76	45.17	132.84
Tryptophan (Trp)	14.80	134.58	14.60	132.77	12.36	112.39	21.28	193.48
Valine (Val)	52.63	150.36	53.86	153.89	52.34	149.53	69.14	197.55
Sulfur AA (Met + Cys)	47.38	189.54	52.92	211.69	52.52	210.09	55.48	221.93
Aromatic AA (Phe + Tyr)	81.83	129.89	79.59	126.34	78.07	123.92	112.03	177.82
Dispensable (DAA)								
Serine (Ser)	47.04		47.94		46.74		58.54	
Arginine (Arg)	106.91		105.91		99.09		129.62	
Glycine (Gly)	58.00		58.44		62.25		62.25	
Aspartic acid (Asp)	105.06		111.21		104.75		147.10	
Glutamic acid (Glu)	261.64		270.89		263.22		322.69	
Alanine (Ala)	43.07		46.03		43.77		57.91	
Proline (Pro)	47.80		48.80		47.08		60.45	
Total IAA	407.51		420.71		409.52		527.93	
Total DAA	669.52		689.22		666.92		838.55	
Total AA (g/100 g protein)	107.70		110.99		107.64		136.65	
First Limiting AA	Lys		Lys		Lys		Lys	

4.2.2.3. In Vitro Protein Digestibility and In Vitro Protein Digestibility Corrected Amino Acid Score

Table 4-4. In vitro protein digestibility (IVPD) and in vitro protein digestibility corrected amino acid score (IV-PDCAAS) for sunflower protein isolates obtained using four extraction methods.

	IVPD (%)	IV-PDCAAS (%)
DES-SFPI	98.49 ± 0.61^{A}	52.66 ± 0.33^{C}
DV-SFPI	98.54 ± 0.08^{A}	62.91 ± 0.16^{A}
MV-SFPI	95.36 ± 0.67^{B}	59.35 ± 0.42^{B}
ALK-SFPI	98.75 ± 0.63^{A}	50.34 ± 0.32^{D}

$^{A-D}$ Superscripts denote a significant difference between samples, $P < 0.05$ using Tukey's test.

The quality of the sunflower protein isolates must be assessed, alongside the amino acid composition, by the extent to which the amino acids undergo hydrolysis during gastrointestinal digestion. As seen in Table *4-4*, there is no significant difference between the in vitro protein digestibility (IVPD) of the DES-SFPI, DV-SFPI, and ALK-SFPI samples, while MV-SFPI had a significantly lower IVPD. This indicates that other extraction methods, such as divalent salt-based and DES extractions, can obtain comparable protein digestibility to the conventional alkaline-based extraction. Although IVPD values were high across all isolates, the in vitro protein digestibility corrected amino acid score (IV-PDCAAS) was significantly different in all SFPI samples, suggesting that it is highly influenced by the indispensable amino acid profile. Lysine was identified as the limiting amino acid in all SFPI samples, therefore, the difference in lysine content likely explains the significant variation in IV-PDCAAS. The DV-SFPI sample had the highest IV-PDCAAS of $62.91 \pm 0.16\%$, followed by MV-SFPI ($59.35 \pm 0.42\%$), DES-SFPI ($52.66 \pm 0.33\%$), and ALK-SFPI with the lowest value of $50.34 \pm 0.32\%$. This suggests that alternative

extraction methods may improve the nutritional value of SFPI compared to alkaline extraction by producing isolates with a well-balanced essential amino acid profile, rather than protein digestibility alone.

Studies in literature have focused on reporting the protein digestibility of SFPI produced using alkaline-based extraction, with studies on other extraction methods being extremely limited. Alexandrino et al. (2017) reported an IVPD value of 90.66 ± 2.64% for alkaline extracted SFPI, which is lower than the digestibility found in this study for ALK-SFPI, but reported a higher PDCAAS of 0.59 (equivalent to 59%). Conversely, the IVPD of ALK-SFPI (98.75 ± 0.63%) is higher than the alkaline extracted sunflower protein concentrates evaluated using a static three-stage in vitro digestion model which reached 82.21% and 94.24% during the gastric and intestinal digestion stages, respectively (X. Huang et al., 2024).

4.3. Conclusion

Overall, the extraction methods significantly influenced the structural properties, amino acid composition, IV-PDCAAS, and anti-nutritional profile of sunflower protein isolates. All SFPI samples maintained the characteristic sunflower protein fractions, including 11S helianthinin and 2S albumins, although differences in band intensity indicated that extraction conditions affected the recovery of certain protein fractions. The four extraction methods also induced different secondary and tertiary structural changes, with MV-SFPI preserving the most ordered structure, while DV-SFPI showed the highest unordered structure content. All isolates exhibited high IVPD values above ranging from 95–98%, demonstrating good digestibility regardless of the extraction method. However, IV-PDCAAS differed significantly, with DV-SFPI having the highest value of 62.91%, compared to the lowest value of 50.34% in ALK-SFPI due to the varying content of the limiting lysine. DES-SFPI showed the lowest TPC of 5.30 mg GAE/g DW, while ALK-SFPI had

the lowest TIA of 0.045 TUI/mg DW. Therefore, the three extraction methods (DES, divalent salt-based, and monovalent salt-based) show potential as more sustainable alternatives for producing SFPI with improved or comparable nutritional quality, IV-PDCAAS, and anti-nutrients content to alkaline-based extraction.

Chapter 5: Conclusions and Future Directions

Overall, this work demonstrated the potential of valorizing commercial sunflower meal (SFM) as a sustainable source for producing sunflower protein isolates (SFPI) using greener extraction technologies. The results from both studies showed that SFM, an industrial by-product of the oil extraction industry, can be used to produce protein ingredients with improved protein purity, techno-functional properties, colour, nutritional quality, and digestibility. This supports the use of SFM as an underutilized plant-based protein source that can contribute to meeting future protein demand while promoting waste reduction, sustainability, and a circular economy. The key findings of this work also show that divalent salt-based, monovalent salt-based, and DES extractions are promising sustainable alternatives to conventional alkaline-based extraction that can be scaled up in the industry. These methods can provide improved or comparable physicochemical, techno-functional, nutritional, and digestibility properties of SFPI, supporting the future use of SFM as a high-value plant protein ingredient for food applications.

5.1. First Research Study (Chapter 3) Key Findings

1. The sustainable extraction methods were more effective than alkaline-based extraction in isolating SFPI with a higher protein purity, extraction yield, and protein recovery rate.
2. MV-SFPI had the highest purity of 90.18%. DES-SFPI had the highest extraction yield and protein recovery rate of 56.92% and 24.73%, respectively.
3. β-sheet structures were the dominant secondary structure in SFM and SFPI samples, followed by α-helix. DV-SFPI retained the highest β-sheet and α-helix content among isolates of 52.93% and 36.74%, respectively, indicating better preservation of ordered secondary structure confirmations. ALK-SFPI had the lowest α-helix content (27.56%) and highest β-turn content (17.83%), indicating greater protein aggregation and rearrangement.

4. The extraction method affected particle size, dispersibility, and aggregation of the isolates. DES-SFPI produced the finest particles, whereas ALK-SFPI exhibited the largest particle sizes values due to aggregation.
5. SEM imaging confirmed that the extraction methods changed the physical structure of SFPI. DV-SFPI showed more granular, small, protein-rich particles, whereas MV-SFPI and DES-SFPI showed larger, plate-like fragments due to the freeze-drying effect. ALK-SFPI showed large, irregular, rough-surfaced aggregates.
6. Colour, a major challenge with sunflower proteins, was greatly improved by the green methods. DV-SFPI was the lightest sample, with the highest L* value of 84.07, while ALK-SFPI was the darkest sample, with the lowest L* value of 65.77. ALK-SFPI also had a negative a* value (-1.13), indicating the development of a green hue in alkaline conditions.
7. Protein solubility was strongly pH-dependent, with low solubility at pH 3 and 5 and higher solubility at pH 7 and 9. At pH 7, MV-SFPI exhibited the highest solubility (47.19%), making it the most suitable isolate for neutral-pH food systems.
8. SFM and ALK-SFPI had the highest water holding capacity, while the green protein extraction methods improved oil holding capacity.
9. Foaming capacity was improved by protein extraction compared to SFM, but the effect depended on pH. DV-SFPI showed the highest foaming capacity at pH 3 (58.33%), pH 5 (77.50%), and pH 7 (45.83%). Foaming stability showed less differences among samples, with high stability at pH 7 and 9, ranging from 82.28–87.50% and 76.08–86.57%, respectively.

10. Emulsification activity index (EAI) was reduced at acidic conditions and improved at higher pH levels of 7 and 9. ALK-SFPI had the highest EAI (9.47 m^2/g) at pH 7, and no significant difference among samples at pH 9.
11. Gelation was strongly pH-dependent and improved under alkaline conditions

5.2. Second Research Study (Chapter 4) Key Findings

1. SDS-PAGE analysis illustrated SFM and SFPI samples with characteristic sunflower protein bands, indicating the presence of 11S helianthinin and 2S albumins across all extraction methods.
2. Near-UV circular dichroism showed that the extraction methods affected the tertiary structure and aromatic amino acid environments of SFPI. ALK-SFPI exhibited the highest positive ellipticity between 260 and 290 nm, suggesting greater aromatic residue rearrangement or exposure under alkaline extraction conditions.
3. SFPI samples had broadly similar amino acid profiles, but the concentrations varied depending on extraction method. The main amino acids in all samples were glutamic acid, aspartic acid, and arginine, with glutamic acid being the most abundant, ranging from 261.64–322.69 mg/g protein. Lysine was the first limiting amino acid in all SFPI samples.
4. All SFPI samples had high in vitro protein digestibility, with no significant difference between samples except for MV-SFPI having a significantly lower result. However, IV-PDCAAS differed significantly among samples due to variation in lysine concentration, the limiting amino acid in all isolates. DV-SFPI had the highest IV-PDCAAS (62.91%), while ALK-SFPI with had the lowest value (50.34%).
5. The extraction methods significantly affected both total phenolic content and trypsin inhibitory activity of SFPI. DES-SFPI had the lowest total phenolic content (5.30 mg

GAE/g DW) and second-lowest trypsin inhibitory activity (0.167 TUI/mg DW). LK-SFPI had the lowest TIA (0.045 TUI/mg DW).

5.3. Future Directions

The findings of this work demonstrated that commercial SFM can be valorized into high-purity SFPI using sustainable extraction technologies. However, further research is needed to support the industrial use of SFPI as food-grade plant protein ingredients. Future work should focus on assessing the scalability of the green extraction methods (divalent salt-based, monovalent salt-based, and DES extractions) through pilot-scale trials. Scale-up studies should evaluate extraction yield, protein recovery, protein purity, water and solvent use, energy requirement, processing time, alternative purification steps (such as membrane or ultrafiltration), and drying efficiency for industrial applicability.

In addition, evaluating the application of the produced SFPI in real food systems is vital. The current research showed that the isolates had promising solubility, foaming, emulsification, water- and oil-holding capacity, and gelation properties, depending on the extraction method and pH. However, these functional properties should be further tested in model and commercial food products, such as plant-based beverages, bakery products, or meat analogs. Blending with other plant-based proteins, such as sources high in lysine, to complement the amino acid profile of SFPI can be investigated in model or commercial food formulations.

More research is also needed to improve the colour and sensory quality of SFPI while maintaining sustainability. While the green extraction methods reduced some of the undesirable effects of CGA associated with alkaline extraction, future studies can investigate mild pre-treatment methods to reduce CGA content prior to protein extraction to further reduce phenolic-

protein interactions. These processes must be suitable for industrial scale-up, sustainable with minimum solvent use, and standardized to address the current gap in CGA pre-treatments.

Moreover, an important future direction is to investigate the reusability and recycling of DES after protein extraction. Although DES extraction showed strong potential for producing SFPI, the method's sustainability depends on the ability to recover and reuse the solvent. After the feasibility of DES recovery is assessed, the composition and water content of the DES should also be monitored after each extraction cycle to determine whether solvent degradation, reduced protein extraction capabilities, or accumulation of non-protein compounds occurs.

References

Achour, J., Guinot, M., Guillon, B., Kapel, R., Galet, O., Adel-Patient, K., Hazebrouck, S., & Bernard, H. (2021). Sensitization potency of sunflower seed protein in a mouse model: Identification of 2S-Albumins more allergenic than SFA-8. *Molecular Nutrition & Food Research*, *65*(18), 2100369. https://doi.org/10.1002/mnfr.202100369

Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nature Protocols*, *2*(4), 875–877. https://doi.org/10.1038/nprot.2007.102

Ajibola, C. F., Fagbemi, T. N., & Aluko, R. E. (2017). Modulation of the secondary and tertiary structures of African yam bean (*S phenostylis stenocarpa)* seed globulins, albumins and protein concentrate by pH and NaCl. *Journal of Food Biochemistry*, *41*(2), e12321. https://doi.org/10.1111/jfbc.12321

Akeson, W. R., & Stahmann, M. A. (1964). A pepsin pancreatin digest index of protein quality evaluation. *The Journal of Nutrition*, *83*(3), 257–261. https://doi.org/10.1093/jn/83.3.257

Alagawany, M., Farag, M. R., El-Hack, M. E. A., & Dhama, K. (2015). The practical application of sunflower meal in poultry nutrition. *Advances in Animal and Veterinary Sciences*, *3*(12), 634–648. https://doi.org/10.14737/journal.aavs/2015/3.12.634.648

Albe Slabi, S., Mathe, C., Basselin, M., Framboisier, X., Ndiaye, M., Galet, O., & Kapel, R. (2020). Multi-objective optimization of solid/liquid extraction of total sunflower proteins from cold press meal. *Food Chemistry*, *317*, 126423. https://doi.org/10.1016/j.foodchem.2020.126423

Albolafio, S., Gil, M. I., Allende, A., & Xanthakis, E. (2021). Potential of wastewater valorization after wet extraction of proteins from faba bean and pea flours. *Recent Progress in Materials*, *03*(02), 1–24. https://doi.org/10.21926/rpm.2102013

Aldalur, A., Devnani, B., Ong, L., & Gras, S. L. (2023). Sustainable plant-based protein sources and their extraction. In *Engineering Plant-Based Food Systems* (pp. 29–60). Elsevier. https://doi.org/10.1016/B978-0-323-89842-3.00004-X

Alexandrino, T. D., Ferrari, R. A., De Oliveira, L. M., De Cássia S.C. Ormenese, R., & Pacheco, M. T. B. (2017). Fractioning of the sunflower flour components: Physical, chemical and nutritional evaluation of the fractions. *LWT*, *84*, 426–432. https://doi.org/10.1016/j.lwt.2017.05.062

Alpiger, S. B., Gregersen, S. B., Ellermann, A. M., Dissing, T. J. J., & Corredig, M. (2025). Extraction of protein-enriched fractions from sunflower and hemp seeds: Composition and colloidal stability of less refined fractions as a function of initial pH. *Future Foods*, *11*, 100541. https://doi.org/10.1016/j.fufo.2025.100541

American Oil Chemists' Society (AOCS). (2017). Trypsin inhibitor activity. In *Official methods and recommended practices* (7th ed.).

AOAC International. (2012). *Official methods of analysis of AOAC international* (W. Horwitz & G. Latimer, Eds.; 19th ed.).

Arakawa, T., Bhat, R., & Timasheff, S. N. (1990). Preferential interactions determine protein solubility in three-component solutions: The magnesium chloride system. *Biochemistry*, *29*(7), 1914–1923. https://doi.org/10.1021/bi00459a036

Arakawa, T., & Timasheff, S. N. (1984). Mechanism of protein salting in and salting out by divalent cation salts: Balance between hydration and salt binding. *Biochemistry*, *23*(25), 5912–5923. https://doi.org/10.1021/bi00320a004

Avilés-Gaxiola, S., Chuck-Hernández, C., Rocha-Pizaña, M. D. R., García-Lara, S., López-Castillo, L. M., & Serna-Saldívar, S. O. (2018). Effect of thermal processing and reducing

agents on trypsin inhibitor activity and functional properties of soybean and chickpea protein concentrates. *LWT*, *98*, 629–634. https://doi.org/10.1016/j.lwt.2018.09.023

Avilés-Gaxiola, S., Chuck-Hernández, C., & Serna Saldívar, S. O. (2018). Inactivation methods of trypsin inhibitor in legumes: A review. *Journal of Food Science*, *83*(1), 17–29. https://doi.org/10.1111/1750-3841.13985

Baker, E. C., & Rackis, J. J. (1986). Preparation of unheated soy protein isolates with low trypsin inhibitor content. In M. Friedman (Ed.), *Nutritional and Toxicological Significance of Enzyme Inhibitors in Foods* (Vol. 199, pp. 349–355). Springer US. https://doi.org/10.1007/978-1-4757-0022-0_20

Bau, H. M., Mohtadi-Nia, D. J., Mejean, L., & Debry, G. (1983). Preparation of colorless sunflower protein products: Effect of processing on physicochemical and nutritional properties. *Journal of the American Oil Chemists' Society*, *60*(6), 1141–1148. https://doi.org/10.1007/BF02671343

Bautista, J., Parrado, J., & Machado, A. (1990). Composition and fractionation of sunflower meal: Use of the lignocellulosic fraction as substrate in solid-state fermentation. *Biological Wastes*, *32*(3), 225–233. https://doi.org/10.1016/0269-7483(90)90051-S

Beaubier, S., Albe-Slabi, S., Aymes, A., Bianeis, M., Galet, O., & Kapel, R. (2021). A rational approach for the production of highly soluble and functional sunflower protein hydrolysates. *Foods*, *10*(3), 664. https://doi.org/10.3390/foods10030664

Beaubier, S., Defaix, C., Albe-Slabi, S., Aymes, A., Galet, O., Fournier, F., & Kapel, R. (2022). Multiobjective decision making strategy for selective albumin extraction from a rapeseed cold-pressed meal based on Rough Set approach. *Food and Bioproducts Processing*, *133*, 34–44. https://doi.org/10.1016/j.fbp.2022.02.005

Berners-Lee, M., Kennelly, C., Watson, R., & Hewitt, C. N. (2018). Current global food production is sufficient to meet human nutritional needs in 2050 provided there is radical societal adaptation. *Elementa: Science of the Anthropocene*, *6*, 52. https://doi.org/10.1525/elementa.310

Bisinotto, M. S., Da Silva Napoli, D. C., Simabuco, F. M., Bezerra, R. M. N., Antunes, A. E. C., Galland, F., & Pacheco, M. T. B. (2023). Sunflower and palm kernel meal present bioaccessible compounds after digestion with antioxidant activity. *Foods*, *12*(17), 3283. https://doi.org/10.3390/foods12173283

Bowen, H., Durrani, R., Delavault, A., Durand, E., Chenyu, J., Yiyang, L., Lili, S., Jian, S., Weiwei, H., & Fei, G. (2022). Application of deep eutectic solvents in protein extraction and purification. *Frontiers in Chemistry*, *10*, 912411. https://doi.org/10.3389/fchem.2022.912411

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., Bohn, T., Bourlieu-Lacanal, C., Boutrou, R., Carrière, F., Clemente, A., Corredig, M., Dupont, D., Dufour, C., Edwards, C., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., … Recio, I. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature Protocols*, *14*(4), 991–1014. https://doi.org/10.1038/s41596-018-0119-1

Bryant, C. M., & McClements, D. J. (2000). Influence of NaCl and $CaCl_2$ on cold-set gelation of heat-denatured whey protein. *Journal of Food Science*, *65*(5), 801–804. https://doi.org/10.1111/j.1365-2621.2000.tb13590.x

Can Karaca, A., Nickerson, M., Caggia, C., Randazzo, C. L., Balange, A. K., Carrillo, C., Gallego, M., Sharifi-Rad, J., Kamiloglu, S., & Capanoglu, E. (2023). Nutritional and functional

properties of novel protein sources. *Food Reviews International*, *39*(9), 6045–6077. https://doi.org/10.1080/87559129.2022.2067174

Carbonaro, M., Maselli, P., & Nucara, A. (2012). Relationship between digestibility and secondary structure of raw and thermally treated legume proteins: A Fourier transform infrared (FT-IR) spectroscopic study. *Amino Acids*, *43*(2), 911–921. https://doi.org/10.1007/s00726-011-1151-4

Carbone, J. W., & Pasiakos, S. M. (2019). Dietary protein and muscle mass: Translating science to application and health benefit. *Nutrients*, *11*(5), 1136. https://doi.org/10.3390/nu11051136

Carré, P. (2021). Reinventing the oilseeds processing to extract oil while preserving the protein. *OCL*, *28*, 13. https://doi.org/10.1051/ocl/2021001

Carré, P., Gärtner, M., Bothe, S., Piofczyk, T., & Hadjiali, S. (2024). Solvent solutions: Comparing extraction methods for edible oils and proteins in a changing regulatory landscape. Part 3: Impacts on the process. *OCL*, *31*, 30. https://doi.org/10.1051/ocl/2024030

Chandran, A. S., Suri, S., & Choudhary, P. (2023). Sustainable plant protein: An up-to-date overview of sources, extraction techniques and utilization. *Sustainable Food Technology*, *1*(4), 466–483. https://doi.org/10.1039/D3FB00003F

Chukwuejim, S., & Aluko, R. E. (2026). Lupin species and protein fraction type affect the conformational state and functional performance of the proteins. *Sustainable Food Proteins*, *4*(2), e70062. https://doi.org/10.1002/sfp2.70062

Church, D. D., Hirsch, K. R., Park, S., Kim, I.-Y., Gwin, J. A., Pasiakos, S. M., Wolfe, R. R., & Ferrando, A. A. (2020). Essential amino acids and protein synthesis: Insights into maximizing the muscle and whole-body response to feeding. *Nutrients*, *12*(12), 3717. https://doi.org/10.3390/nu12123717

Colgrave, M. L., Korsinczky, M. J. L., Clark, R. J., Foley, F., & Craik, D. J. (2010). Sunflower trypsin inhibitor-1, proteolytic studies on a trypsin inhibitor peptide and its analogs. *Peptide Science*, *94*(5), 665–672. https://doi.org/10.1002/bip.21415

Cravotto, C., Fabiano-Tixier, A.-S., Claux, O., Abert-Vian, M., Tabasso, S., Cravotto, G., & Chemat, F. (2022). Towards substitution of hexane as extraction solvent of food products and ingredients with no regrets. *Foods*, *11*(21), 3412. https://doi.org/10.3390/foods11213412

Damodaran, S. (2005). Protein stabilization of emulsions and foams. *Journal of Food Science*, *70*(3). https://doi.org/10.1111/j.1365-2621.2005.tb07150.x

Dauguet, S., Labalette, F., Fine, F., Carré, P., Merrien, A., & Palleau, J.-P. (2016). Genetic impact on protein content and hullability of sunflower seeds, and on the quality of sunflower meal. *OCL*, *23*(2), D205. https://doi.org/10.1051/ocl/2016003

De Oliveira Filho, J. G., & Egea, M. B. (2021). Sunflower seed byproduct and its fractions for food application: An attempt to improve the sustainability of the oil process. *Journal of Food Science*, *86*(5), 1497–1510. https://doi.org/10.1111/1750-3841.15719

Đermanović, B., Marić, A., Sakač, M., Tomić, D., Dragojlović, D., Popović, L., Šarić, B., & Jovanov, P. (2025). Impact of alkaline and deep eutectic solvent extraction on rapeseed protein isolates characteristics and in vitro digestibility. *Current Research in Food Science*, *10*, 101075. https://doi.org/10.1016/j.crfs.2025.101075

Dimina, L., Rémond, D., Huneau, J.-F., & Mariotti, F. (2022). Combining plant proteins to achieve amino acid profiles adapted to various nutritional objectives—An exploratory analysis using linear programming. *Frontiers in Nutrition*, *8*, 809685. https://doi.org/10.3389/fnut.2021.809685

Düsterhöft, E., Posthumus, M. A., & Voragen, A. G. J. (1992). Non-starch polysaccharides from sunflower (*Helianthus annuus*) meal and palm-kernel (*Elaeis guineensis*) meal—Investigation of the structure of major polysaccharides. *Journal of the Science of Food and Agriculture*, *59*(2), 151–160. https://doi.org/10.1002/jsfa.2740590204

Düsterhöft, E., Voragen, A. G. J., & Engels, F. M. (1991). Non-starch polysaccharides from sunflower (*Helianthus annuus*) meal and palm kernel (*Elaeis guineenis*) meal—Preparation of cell wall material and extraction of polysaccharide fractions. *Journal of the Science of Food and Agriculture*, *55*(3), 411–422. https://doi.org/10.1002/jsfa.2740550309

Foegeding, E. A., & Davis, J. P. (2011). Food protein functionality: A comprehensive approach. *Food Hydrocolloids*, *25*(8), 1853–1864. https://doi.org/10.1016/j.foodhyd.2011.05.008

Food and Agriculture Organization of the United Nations (FAO) & World Health Organization (WHO). (1991). *Protein quality evaluation: Report of the joint FAO/WHO expert consultation*. *51*(51).

Frota, K. D. M. G., Lopes, L. A. R., Silva, I. C. V., & Arêas, J. A. G. (2018). Molecular profile, purity and presence of trypsin inhibitors in cowpea protein isolates. *Revista Caatinga*, *31*(1), 202–208. https://doi.org/10.1590/1983-21252018v31n123rc

González-Pérez, S., Gerrit A. Van Konignsveld, Vereijken, J. M., Merck, K. B., Gruppen, H., & Voragen, A. G. J. (2005). Emulsion properties of sunflower (*Helianthus annuus*) proteins. *Journal of Agricultural and Food Chemistry*, *53*(6), 2261–2267. https://doi.org/10.1021/jf0486388

González-Pérez, S., Merck, K. B., Vereijken, J. M., Van Koningsveld, G. A., Gruppen, H., & Voragen, A. G. J. (2002). Isolation and characterization of undenatured chlorogenic acid

free sunflower (*Helianthus annuus*) proteins. *Journal of Agricultural and Food Chemistry*, *50*(6), 1713–1719. https://doi.org/10.1021/jf011245d

González-Pérez, S., & Vereijken, J. M. (2007). Sunflower proteins: Overview of their physicochemical, structural and functional properties. *Journal of the Science of Food and Agriculture*, *87*(12), 2173–2191. https://doi.org/10.1002/jsfa.2971

González-Pérez, S., Vereijken, J. M., Merck, K. B., Van Koningsveld, G. A., Gruppen, H., & Voragen, A. G. J. (2004). Conformational states of sunflower (*Helianthus annuus*) helianthinin: Effect of heat and pH. *Journal of Agricultural and Food Chemistry*, *52*(22), 6770–6778. https://doi.org/10.1021/jf049612j

Grahovac, N., Aleksić, M., Trajkovska, B., Marjanović Jeromela, A., & Nakov, G. (2025). Extraction and valorization of oilseed cakes for value-added food components—A review for a sustainable foodstuff production in a case process approach. *Foods*, *14*(13), 2244. https://doi.org/10.3390/foods14132244

Grela, E. R., Kiczorowska, B., Samolińska, W., Matras, J., Kiczorowski, P., Rybiński, W., & Hanczakowska, E. (2017). Chemical composition of leguminous seeds: Part I—content of basic nutrients, amino acids, phytochemical compounds, and antioxidant activity. *European Food Research and Technology*, *243*(8), 1385–1395. https://doi.org/10.1007/s00217-017-2849-7

Grudniewska, A., De Melo, E. M., Chan, A., Gniłka, R., Boratyński, F., & Matharu, A. S. (2018). Enhanced protein extraction from oilseed cakes using glycerol–choline chloride deep eutectic solvents: A biorefinery approach. *ACS Sustainable Chemistry & Engineering*, *6*(11), 15791–15800. https://doi.org/10.1021/acssuschemeng.8b04359

Gültekin Subaşı, B., Vahapoğlu, B., Capanoglu, E., & Mohammadifar, M. A. (2022). A review on protein extracts from sunflower cake: Techno-functional properties and promising modification methods. *Critical Reviews in Food Science and Nutrition*, *62*(24), 6682–6697. https://doi.org/10.1080/10408398.2021.1904821

Hadidi, M., Aghababaei, F., & McClements, D. J. (2024). Sunflower meal/cake as a sustainable protein source for global food demand: Towards a zero-hunger world. *Food Hydrocolloids*, *147*, 109329. https://doi.org/10.1016/j.foodhyd.2023.109329

Hallouch, O., Ibourki, M., Asbbane, A., Devkota, K., Giuffrè, A. M., Majourhat, K., & Gharby, S. (2025). Exploring the bioactive potential of argan oil cake: A comprehensive physicochemical comparison with various seeds cakes. *Current Research in Nutrition and Food Science Journal*, *13*(1), 77–100. https://doi.org/10.12944/CRNFSJ.13.1.5

Henchion, M., Hayes, M., Mullen, A., Fenelon, M., & Tiwari, B. (2017). Future protein supply and demand: Strategies and factors influencing a sustainable equilibrium. *Foods*, *6*(7), 53. https://doi.org/10.3390/foods6070053

Hewage, A., Olatunde, O. O., Nimalaratne, C., House, J. D., Aluko, R. E., & Bandara, N. (2024). Improved protein extraction technology using deep eutectic solvent system for producing high purity fava bean protein isolates at mild conditions. *Food Hydrocolloids*, *147*, 109283. https://doi.org/10.1016/j.foodhyd.2023.109283

Hewage, A., Olatunde, O. O., Nimalaratne, C., Malalgoda, M., Aluko, R. E., & Bandara, N. (2022). Novel Extraction technologies for developing plant protein ingredients with improved functionality. *Trends in Food Science & Technology*, *129*, 492–511. https://doi.org/10.1016/j.tifs.2022.10.016

Hsu, H. W., Vavak, D. L., Satterlee, L. D., & Miller, G. A. (1977). A multienzyme technique for estimating protein digestibility. *Journal of Food Science*, *42*(5), 1269–1273. https://doi.org/10.1111/j.1365-2621.1977.tb14476.x

Huang, J., Xie, M., He, L., Song, X., & Cao, T. (2023). Chlorogenic acid: A review on its mechanisms of anti-inflammation, disease treatment, and related delivery systems. *Frontiers in Pharmacology*, *14*, 1218015. https://doi.org/10.3389/fphar.2023.1218015

Huang, X., Li, Y., Cui, C., & Sun-Waterhouse, D. (2024). Structural, functional properties, and in vitro digestibility of sunflower protein concentrate as affected by extraction method: Isoelectric precipitation vs ultrafiltration. *Food Chemistry*, *439*, 138090. https://doi.org/10.1016/j.foodchem.2023.138090

Hunter, M. C., Smith, R. G., Schipanski, M. E., Atwood, L. W., & Mortensen, D. A. (2017). Agriculture in 2050: Recalibrating targets for sustainable intensification. *BioScience*, *67*(4), 386–391. https://doi.org/10.1093/biosci/bix010

International Organization for Standardization (ISO). (2005). *ISO 13904:2005—Animal feeding stuffs—Determination of tryptophan content*. https://www.iso.org/standard/37259.html

Iqbal, A., Khalil, I. A., Ateeq, N., & Sayyar Khan, M. (2006). Nutritional quality of important food legumes. *Food Chemistry*, *97*(2), 331–335. https://doi.org/10.1016/j.foodchem.2005.05.011

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

Ismail, B. P., Senaratne-Lenagala, L., Stube, A., & Brackenridge, A. (2020). Protein demand: Review of plant and animal proteins used in alternative protein product development and production. *Animal Frontiers*, *10*(4), 53–63. https://doi.org/10.1093/af/vfaa040

Ivanova, P., Chalova, V., & Koleva, L. (2014). Functional properties of proteins isolated from industrially produced sunflower meal. *International Journal of Food Studies*, *3*(2), 203–212. https://doi.org/10.7455/ijfs/3.2.2014.a6

Ivanova, P., Chalova, V., Koleva, L., & Pishtiyski, I. (2013). Amino acid composition and solubility of proteins isolated from sunflower meal produced in Bulgaria. *International Food Research Journal*, *20*(6), 2995.

Ivanova, P., Chalova, V., Koleva, L., Pishtiyski, I., & Perifanova-Nemska, M. (2012). Optimization of protein extraction from sunflower meal produced in Bulgaria. *Bulgarian Journal of Agricultural Science*, *18*(2), 153–160.

Jia, W., Sethi, D. S., Van Der Goot, A. J., & Keppler, J. K. (2022). Covalent and non-covalent modification of sunflower protein with chlorogenic acid: Identifying the critical ratios that affect techno-functionality. *Food Hydrocolloids*, *131*, 107800. https://doi.org/10.1016/j.foodhyd.2022.107800

Kaczmarek, D., Pokora-Carzynska, M., Juszczak, L., Jamroz, E., & Kapusniak, J. (2025). Plant proteins as alternative natural emulsifiers in food emulsions. *Foods*, *14*(24), 4291. https://doi.org/10.3390/foods14244291

Karabulut, G., Sanli, I., & Ozkan, G. (2025). Green extraction using natural deep eutectic solvents for the recovery of proteins from sunflower meal. *International Journal of Biological Macromolecules*, *328*, 147556. https://doi.org/10.1016/j.ijbiomac.2025.147556

Karaca, A. C., Low, N., & Nickerson, M. (2011). Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. *Food Research International*, *44*(9), 2742–2750. https://doi.org/10.1016/j.foodres.2011.06.012

Karamać, M., Kosińska, A., Estrella, I., Hernández, T., & Dueñas, M. (2012). Antioxidant activity of phenolic compounds identified in sunflower seeds. *European Food Research and Technology*, *235*(2), 221–230. https://doi.org/10.1007/s00217-012-1751-6

Karefyllakis, D., Altunkaya, S., Berton-Carabin, C. C., Van Der Goot, A. J., & Nikiforidis, C. V. (2017). Physical bonding between sunflower proteins and phenols: Impact on interfacial properties. *Food Hydrocolloids*, *73*, 326–334. https://doi.org/10.1016/j.foodhyd.2017.07.018

Kaur, R., & 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

Kelly, S. M., Jess, T. J., & Price, N. C. (2005). How to study proteins by circular dichroism. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, *1751*(2), 119–139. https://doi.org/10.1016/j.bbapap.2005.06.005

Khalesi, M., Glenn-Davi, K., Mohammadi, N., & FitzGerald, R. J. (2024). Key factors influencing gelation in plant vs. animal proteins: A comparative mini-review. *Gels*, *10*(9), 575. https://doi.org/10.3390/gels10090575

Khalil, M., Ragab, M., & Hassanien, F. R. (1985). Some functional properties of oilseed proteins. *Food / Nahrung*, *29*(3), 275–282. https://doi.org/10.1002/food.19850290312

Kim, S., & Kirshnan, H. (2023). A fast and cost-effective procedure for reliable measurement of trypsin inhibitor activity in soy and soy products. In *Methods in Enzymology* (Vol. 680, pp. 195–213). Elsevier. https://doi.org/10.1016/bs.mie.2022.08.016

Konarev, Al. V., Anisimova, I. N., Gavrilova, V. A., Rozhkova, V. T., Fido, R., Tatham, A. S., & Shewry, P. R. (2000). Novel proteinase inhibitors in seeds of sunflower (Helianthus annuus L.): Polymorphism, inheritance and properties. *Theoretical and Applied Genetics*, *100*(1), 82–88. https://doi.org/10.1007/s001220050012

Korsinczky, M. L. J., Schirra, H. J., Rosengren, K. J., West, J., Condie, B. A., Otvos, L., Anderson, M. A., & Craik, D. J. (2001). Solution structures by H-1 NMR of the novel cyclic trypsin inhibitor SFTI-1 from sunflower seeds and an acyclic permutant. *Journal of Molecular Biology*, *311*(3), 579–591. https://doi.org/10.1006/jmbi.2001.4887

Lakemond, C. M. M., De Jongh, H. H. J., Hessing, M., Gruppen, H., & Voragen, A. G. J. (2000). Heat denaturation of soy glycinin: Influence of pH and ionic strength on molecular structure. *Journal of Agricultural and Food Chemistry*, *48*(6), 1991–1995. https://doi.org/10.1021/jf9908704

Lan, T., Dong, Y., Jiang, L., Zhang, Y., & Sui, X. (2024). Analytical approaches for assessing protein structure in protein-rich food: A comprehensive review. *Food Chemistry: X*, *22*, 101365. https://doi.org/10.1016/j.fochx.2024.101365

Lavine, E., & Ben-Shoshan, M. (2015). Allergy to sunflower seed and sunflower butter as proposed vehicle for sensitization. *Allergy, Asthma & Clinical Immunology*, *11*(1), 2. https://doi.org/10.1186/s13223-014-0065-6

Le Clef, E., & Kemper, T. (2015). Sunflower seed preparation and oil extraction. In *Sunflower* (pp. 187–226). Elsevier. https://doi.org/10.1016/B978-1-893997-94-3.50014-3

Li, P., Yin, Y.-L., Li, D., Woo Kim, S., & Wu, G. (2007). Amino acids and immune function. *British Journal of Nutrition*, *98*(2), 237–252. https://doi.org/10.1017/S000711450769936X

Li, Z., Xiang, F., Huang, X., Liang, M., Ma, S., Gafurov, K., Gu, F., Guo, Q., & Wang, Q. (2024). Properties and characterization of sunflower seeds from different varieties of edible and oil sunflower seeds. *Foods*, *13*(8), 1188. https://doi.org/10.3390/foods13081188

Ling, J. K. U., & Hadinoto, K. (2022). Deep eutectic solvent as green solvent in extraction of biological macromolecules: A Review. *International Journal of Molecular Sciences*, *23*(6), 3381. https://doi.org/10.3390/ijms23063381

Liu, H.-M., Liu, X.-Y., Yan, Y.-Y., Gao, J.-H., Qin, Z., & Wang, X.-D. (2021). Structural properties and antioxidant activities of polysaccharides isolated from sunflower meal after oil extraction. *Arabian Journal of Chemistry*, *14*(12), 103420. https://doi.org/10.1016/j.arabjc.2021.103420

Lomascolo, A., Uzan-Boukhris, E., Sigoillot, J.-C., & Fine, F. (2012). Rapeseed and sunflower meal: A review on biotechnology status and challenges. *Applied Microbiology and Biotechnology*, *95*(5), 1105–1114. https://doi.org/10.1007/s00253-012-4250-6

Luckett, S., Garcia, R. S., Barker, J. J., Konarev, Al. V., Shewry, P. R., Clarke, A. R., & Brady, R. L. (1999). High-resolution structure of a potent, cyclic proteinase inhibitor from sunflower seeds. *Journal of Molecular Biology*, *290*(2), 525–533. https://doi.org/10.1006/jmbi.1999.2891

Ma, K. K., Greis, M., Lu, J., Nolden, A. A., McClements, D. J., & Kinchla, A. J. (2022). Functional performance of plant proteins. *Foods*, *11*(4), 594. https://doi.org/10.3390/foods11040594

Ma, Y., & Chen, F. (2023). Plant protein heat-induced gels: Formation mechanisms and regulatory strategies. *Coatings*, *13*(11), 1899. https://doi.org/10.3390/coatings13111899

Malik, M. A., & Saini, C. S. (2017). Polyphenol removal from sunflower seed and kernel: Effect on functional and rheological properties of protein isolates. *Food Hydrocolloids*, *63*, 705–715. https://doi.org/10.1016/j.foodhyd.2016.10.026

Malik, M. A., Sharma, H. K., & Saini, C. S. (2016). Effect of removal of phenolic compounds on structural and thermal properties of sunflower protein isolate. *Journal of Food Science and Technology*, *53*(9), 3455–3464. https://doi.org/10.1007/s13197-016-2320-y

Malik, M. A., Sharma, H. K., & Saini, C. S. (2017). High intensity ultrasound treatment of protein isolate extracted from dephenolized sunflower meal: Effect on physicochemical and functional properties. *Ultrasonics Sonochemistry*, *39*, 511–519. https://doi.org/10.1016/j.ultsonch.2017.05.026

Malomo, S. A., He, R., & Aluko, R. E. (2014). Structural and functional properties of hemp seed protein products. *Journal of Food Science*, *79*(8). https://doi.org/10.1111/1750-3841.12537

Manitoba Agriculture. (2025). *Sunflowers: Sector profile at a glance [PDF]*. Government of Manitoba. https://www.gov.mb.ca/agriculture/markets-and-statistics/crop-statistics/pubs/sunflower-profile-2025.pdf

Marinangeli, C. P. F., & House, J. D. (2017). Potential impact of the digestible indispensable amino acid score as a measure of protein quality on dietary regulations and health. *Nutrition Reviews*, *75*(8), 658–667. https://doi.org/10.1093/nutrit/nux025

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., … Brodkorb, A. (2014). A standardised static *in vitro* digestion method suitable for food – An international consensus. *Food Funct.*, *5*(6), 1113–1124. https://doi.org/10.1039/C3FO60702J

Mohammed, K., Obadi, M., Omedi, M., Letsididi, K. S., Koko, M., Zaaboul, F., & Liu, Y. (2018). Effect of sunflower meal protein isolate (SMPI) addition on wheat bread quality. *Journal of Academia and Industrial Research*, *6*(9), 159–164.

Molina, M. I., Petruccelli, S., & Añón, M. C. (2004). Effect of pH and ionic strength modifications on thermal denaturation of the 11S globulin of sunflower (*Helianthus annuus*). *Journal of Agricultural and Food Chemistry*, *52*(19), 6023–6029. https://doi.org/10.1021/jf0494175

Mosenthin, R., Messerschmidt, U., Sauer, N., Carré, P., Quinsac, A., & Schöne, F. (2016). Effect of the desolventizing/toasting process on chemical composition and protein quality of rapeseed meal. *Journal of Animal Science and Biotechnology*, *7*(1), 36. https://doi.org/10.1186/s40104-016-0095-7

Muhammad, H., Ali, S. F., Ahmad, I., & Sadiq, M. B. (2026). Optimized extraction and comprehensive characterization of sunflower meal protein for functional application in plant-based meat analogs. *Discover Food*, *6*(1), 177. https://doi.org/10.1007/s44187-026-00869-1

Murru, M., & Calvo, C. L. (2020). Sunflower protein enrichment. Methods and potential applications. *OCL*, *27*, 17. https://doi.org/10.1051/ocl/2020007

Nadeeshani, H., Hewage, A., Zhao, Y., Sá, A. G. A., House, J. D., & Bandara, N. (2026). Structure-functional properties and protein quality of climate-smart white lupin (Lupinus albus) protein isolates: Impact of deep eutectic solvent and conventional protein extraction methods. *Food Hydrocolloids*, *175*, 112528. https://doi.org/10.1016/j.foodhyd.2026.112528

Nde, D., & Foncha, A. (2020). Optimization methods for the extraction of vegetable oils: A review. *Processes*, *8*(2), 209. https://doi.org/10.3390/pr8020209

Nevara, G. A., Giwa Ibrahim, S., Syed Muhammad, S. K., Zawawi, N., Mustapha, N. A., & Karim, R. (2023). Oilseed meals into foods: An approach for the valorization of oilseed by-products. *Critical Reviews in Food Science and Nutrition*, *63*(23), 6330–6343. https://doi.org/10.1080/10408398.2022.2031092

Nguyen, V., Taine, E. G., Meng, D., Cui, T., & Tan, W. (2024). Chlorogenic acid: A systematic review on the biological functions, mechanistic actions, and therapeutic potentials. *Nutrients*, *16*(7), 924. https://doi.org/10.3390/nu16070924

Oseyko, M., Romanovska, T., & Shevchyk, V. (2020). Justification of the amino acid composition of sunflower proteins for dietary and functional products. *Ukrainian Food Journal*, *9*(2), 394–403. https://doi.org/10.24263/2304-974X-2020-9-2-11

Öztürk, Z., Lille, M., Rosa-Sibakov, N., & Sozer, N. (2024). Impact of heat treatment and high moisture extrusion on the in vitro protein digestibility of sunflower and pea protein ingredients. *LWT*, *214*, 117133. https://doi.org/10.1016/j.lwt.2024.117133

Parodi, E., La Nasa, J., Ribechini, E., Petri, A., & Piccolo, O. (2023). Extraction of proteins and residual oil from flax (Linum usitatissimum), camelina (Camelina sativa), and sunflower (Helianthus annuus) oilseed press cakes. *Biomass Conversion and Biorefinery*, *13*(3), 1915–1926. https://doi.org/10.1007/s13399-021-01379-z

Patel, P., Pang, Y. L. J., Choi, W. J., & Wong, A. (2025). Protein extraction and isolation from legumes and algae: An industry primer. *Food and Bioprocess Technology*, *18*(10), 8380–8408. https://doi.org/10.1007/s11947-025-03958-8

Pedersen, B., & Eggum, B. O. (1981). Prediction of protein digestibility by in vitro procedures based on two multi-enzyme systems. *Zeitschrift Für Tierphysiologie Tierernährung Und Futtermittelkunde*, *45*(1–5), 190–200. https://doi.org/10.1111/j.1439-0396.1981.tb01319.x

Petraru, A., Ursachi, F., & Amariei, S. (2021). Nutritional characteristics assessment of sunflower seeds, oil and cake. Perspective of using sunflower oilcakes as a functional ingredient. *Plants*, *10*(11), 2487. https://doi.org/10.3390/plants10112487

Pickardt, C., Neidhart, S., Griesbach, C., Dube, M., Knauf, U., Kammerer, D. R., & Carle, R. (2009). Optimisation of mild-acidic protein extraction from defatted sunflower (Helianthus annuus L.) meal. *Food Hydrocolloids*, *23*(7), 1966–1973. https://doi.org/10.1016/j.foodhyd.2009.02.001

Pillai, R. R., & Kurpad, A. V. (2012). Amino acid requirements in children and the elderly population. *British Journal of Nutrition*, *108*(S2), S44–S49. https://doi.org/10.1017/S0007114512002401

Pilorgé, E. (2020). Sunflower in the global vegetable oil system: Situation, specificities and perspectives. *OCL*, *27*, 34. https://doi.org/10.1051/ocl/2020028

Ren, J., Song, C.-L., Wang, P., Li, S., Kopparapu, N.-K., & Zheng, X.-Q. (2015). Modification of structural and functional properties of sunflower 11S globulin hydrolysates. *Czech Journal of Food Sciences*, *33*(5), 474–479. https://doi.org/10.17221/154/2015-CJFS

Rente, D., Paiva, A., & Duarte, A. R. (2021). The role of hydrogen bond donor on the extraction of phenolic compounds from natural matrices using deep eutectic systems. *Molecules*, *26*(8), 2336. https://doi.org/10.3390/molecules26082336

Rivera-Najera, L. Y., Saab-Rincón, G., Battaglia, M., Amero, C., Pulido, N. O., García-Hernández, E., Solórzano, R. M., Reyes, J. L., & Covarrubias, A. A. (2014). A group 6 late embryogenesis abundant protein from common bean is a disordered protein with extended helical structure and oligomer-forming properties. *Journal of Biological Chemistry*, *289*(46), 31995–32009. https://doi.org/10.1074/jbc.M114.583369

Rodriguezpatino, J., Minonesconde, J., Linares, H., Pedrochejimenez, J., Carrerasanchez, C., Pizones, V., & Rodriguez, F. (2007). Interfacial and foaming properties of enzyme-induced hydrolysis of sunflower protein isolate. *Food Hydrocolloids*, *21*(5–6), 782–793. https://doi.org/10.1016/j.foodhyd.2006.09.002

Roy, D. N., & Bhat, R. V. (1974). Trypsin inhibitor content in some varieties of soya bean (*Glycine max* L.) and sunflower seeds (*Helianthus annuus* L.). *Journal of the Science of Food and Agriculture*, *25*(7), 765–769. https://doi.org/10.1002/jsfa.2740250704

Saeed, M., & Cheryan, M. (1988). Sunflower protein concentrates and isolates' low in polyphenols and phytate. *Journal of Food Science*, *53*(4), 1127–1131. https://doi.org/10.1111/j.1365-2621.1988.tb13545.x

Saleh, A. A., El-Awady, A., Amber, K., Eid, Y. Z., Alzawqari, M. H., Selim, S., Soliman, M. M., & Shukry, M. (2021). Effects of sunflower meal supplementation as a complementary protein source in the laying hen's diet on productive performance, egg quality, and nutrient digestibility. *Sustainability*, *13*(6), 3557. https://doi.org/10.3390/su13063557

Salgado, P. R., Drago, S. R., Molina Ortiz, S. E., Petruccelli, S., Andrich, O., González, R. J., & Mauri, A. N. (2012). Production and characterization of sunflower (Helianthus annuus L.) protein-enriched products obtained at pilot plant scale. *LWT - Food Science and Technology*, *45*(1), 65–72. https://doi.org/10.1016/j.lwt.2011.07.021

Salgado, P. R., Molina Ortiz, S. E., Petruccelli, S., & 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

Salter, A. M., & Lopez-Viso, C. (2021). Role of novel protein sources in sustainably meeting future global requirements. *Proceedings of the Nutrition Society*, *80*(2), 186–194. https://doi.org/10.1017/S0029665121000513

Samtiya, M., Aluko, R. E., & Dhewa, T. (2020). Plant food anti-nutritional factors and their reduction strategies: An overview. *Food Production, Processing and Nutrition*, *2*(1), 6. https://doi.org/10.1186/s43014-020-0020-5

Sara, A. S., Mathé, C., Basselin, M., Fournier, F., Aymes, A., Bianeis, M., Galet, O., & 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

Saricaoglu, B., Yılmaz, H., Subaşı, B. G., & Capanoglu, E. (2023). Effect of de-phenolization on protein-phenolic interactions of sunflower protein isolate. *Food Research International*, *164*, 112345. https://doi.org/10.1016/j.foodres.2022.112345

Sarwar Gilani, G., Wu Xiao, C., & Cockell, K. A. (2012). Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. *British Journal of Nutrition*, *108*(S2), S315–S332. https://doi.org/10.1017/S0007114512002371

Schaafsma, G. (2000). The protein digestibility–corrected amino acid score. *The Journal of Nutrition*, *130*(7), 1865S-1867S. https://doi.org/10.1093/jn/130.7.1865S

Schmidt, F., Blankart, M., Wanger, J., Scharfe, M., Scheuerer, T., & Hinrichs, J. (2022). Upscaling of alkaline pea protein extraction from dry milled and pre-treated peas from laboratory to pilot scale: Optimization of process parameters for higher protein yields. *Journal of Food*

Measurement and Characterization, *16*(6), 4904–4913. https://doi.org/10.1007/s11694-022-01558-0

Sharma, V., Tsai, M.-L., Sun, P.-P., Chen, C.-W., Nargotra, P., & Dong, C.-D. (2023). Sequential ultrasound assisted deep eutectic solvent-based protein extraction from Sacha inchi meal biomass: Towards circular bioeconomy. *Journal of Food Science and Technology*, *60*(4), 1425–1434. https://doi.org/10.1007/s13197-023-05689-0

Shchekoldina, T., & Aider, M. (2014). Production of low chlorogenic and caffeic acid containing sunflower meal protein isolate and its use in functional wheat bread making. *Journal of Food Science and Technology*, *51*(10), 2331–2343. https://doi.org/10.1007/s13197-012-0780-2

Singh, R., Sá, A. G. A., Sharma, S., Nadimi, M., Paliwal, J., House, J. D., & Koksel, F. (2024). Effects of feed moisture content on the physical and nutritional quality attributes of sunflower meal-based high-moisture meat analogues. *Food and Bioprocess Technology*, *17*(7), 1897–1913. https://doi.org/10.1007/s11947-023-03225-8

Smith, K., Watson, A. W., Lonnie, M., Peeters, W. M., Oonincx, D., Tsoutsoura, N., Simon-Miquel, G., Szepe, K., Cochetel, N., Pearson, A. G., Witard, O. C., Salter, A. M., Bennett, M., & Corfe, B. M. (2024). Meeting the global protein supply requirements of a growing and ageing population. *European Journal of Nutrition*, *63*(5), 1425–1433. https://doi.org/10.1007/s00394-024-03358-2

Statistics Canada. (2024). *Estimated areas, yield, production, average farm price and total farm value of principal field crops, in metric and imperial units.* https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=3210035901

Statistics Canada. (2026). *Estimated areas, yield, production, average farm price and total farm value of principal field crops, in metric and imperial units*. https://doi.org/https://doi.org/10.25318/3210035901-eng

Subaşı, B. G., Casanova, F., Capanoglu, E., Ajalloueian, F., Sloth, J. J., & Mohammadifar, M. A. (2020). Protein extracts from de-oiled sunflower cake: Structural, physico-chemical and functional properties after removal of phenolics. *Food Bioscience*, *38*, 100749. https://doi.org/10.1016/j.fbio.2020.100749

Taha, F. S., Mohamed, G. F., Mohamed, S. H., Mohamed, S. S., & Kamil, M. M. (2011). Optimization of the extraction of total phenolic compounds from sunflower meal and evaluation of the bioactivities of chosen extracts. *American Journal of Food Technology*, *6*(12), 1002–1020. https://doi.org/10.3923/ajft.2011.1002.1020

Tajik, N., Tajik, M., Mack, I., & Enck, P. (2017). The potential effects of chlorogenic acid, the main phenolic components in coffee, on health: A comprehensive review of the literature. *European Journal of Nutrition*, *56*(7), 2215–2244. https://doi.org/10.1007/s00394-017-1379-1

Tang, J., Wichers, H. J., & Hettinga, K. A. (2022). Heat-induced unfolding facilitates plant protein digestibility during in vitro static infant digestion. *Food Chemistry*, *375*, 131878. https://doi.org/10.1016/j.foodchem.2021.131878

Tessier, R., Khodorova, N., Calvez, J., Kapel, R., Quinsac, A., Piedcoq, J., Tomé, D., & Gaudichon, C. (2020). 15N and 2H intrinsic labeling demonstrate that real digestibility in rats of proteins and amino acids from sunflower protein isolate is almost as high as that of goat whey. *The Journal of Nutrition*, *150*(3), 450–457. https://doi.org/10.1093/jn/nxz279

Thapa, S., Brownstein, K., McTaggart, R., & Karki, B. (2025). Mechanistic insights into enhancement of sunflower protein isolates through a combined fractionation and fungal bioprocessing of sunflower meal. *LWT*, *238*, 118846. https://doi.org/10.1016/j.lwt.2025.118846

Tintor, Đ., Ninković, K., Milošević, J., & Polović, N. Đ. (2024). Gaining insight into protein structure via ATR-FTIR spectroscopy. *Vibrational Spectroscopy*, *134*, 103726. https://doi.org/10.1016/j.vibspec.2024.103726

Tinus, T., Damour, M., Van Riel, V., & Sopade, P. A. (2012). Particle size-starch–protein digestibility relationships in cowpea (Vigna unguiculata). *Journal of Food Engineering*, *113*(2), 254–264. https://doi.org/10.1016/j.jfoodeng.2012.05.041

Tiong, A. Y. J., Shrestha, S., Attenborough, E., Michalski, P., Crawford, S., Batchelor, W., & Van 'T Hag, L. (2026). The effect of legume protein source, solubility, pH, and salt addition on the texture of meat analogues. *LWT*, *242*, 118956. https://doi.org/10.1016/j.lwt.2025.118956

Totosaus, A., Montejano, J. G., Salazar, J. A., & Guerrero, I. (2002). A review of physical and chemical protein-gel induction. *International Journal of Food Science and Technology*, *37*(6), 589–601. https://doi.org/10.1046/j.1365-2621.2002.00623.x

Treviño, J., Rebolé, A., Rodríguez, M. L., Ortiz, L. T., Centeno, C., & Alzueta, C. (1998). Nutritional effect of chlorogenic acid fed to growing broiler chicks. *Journal of the Science of Food and Agriculture*, *76*(2), 156–160. https://doi.org/10.1002/(SICI)1097-0010(199802)76:2%3C156::AID-JSFA912%3E3.0.CO;2-M

United Nations Department of Economic and Social Affairs. (2024). *World population prospects 2024: Summary of results*. https://population.un.org/wpp/?utm_source=chatgpt.com

USDA-Economic Research Service. (2026). *Oil crops yearbook*. https://www.ers.usda.gov/data-products/oil-crops-yearbook

USDA-FAS. (2026). *Oilseeds: World market and trade* [Global Market Analysis, June 2026]. United States Department of Agriculture - Foreign Agricultural Service.

Vidosavljević, S., Bojanić, N., Ilić, P., Rakić, D., Đuragić, O., Banjac, V., & Fišteš, A. (2022). Optimization of grinding process of sunflower meal for obtaining protein-enriched fractions. *Processes*, *10*(12), 2704. https://doi.org/10.3390/pr10122704

Villamide, M. J., & San Juan, L. D. (1998). Effect of chemical composition of sunflower seed meal on its true metabolizable energy and amino acid digestibility. *Poultry Science*, *77*(12), 1884–1892. https://doi.org/10.1093/ps/77.12.1884

Vogelsang-O'Dwyer, M., Petersen, I. L., Joehnke, M. S., Sørensen, J. C., Bez, J., Detzel, A., Busch, M., Krueger, M., O'Mahony, J. A., Arendt, E. K., & Zannini, E. (2020). Comparison of faba bean protein ingredients produced using dry fractionation and isoelectric precipitation: Techno-functional, nutritional and environmental performance. *Foods*, *9*(3), 322. https://doi.org/10.3390/foods9030322

Wang, H., Tong, X., Yuan, Y., Peng, X., Zhang, Q., Zhang, S., Xie, C., Zhang, X., Yan, S., Xu, J., Jiang, L., Qi, B., & Li, Y. (2020). Effect of spray-drying and freeze-drying on the properties of soybean hydrolysates. *Journal of Chemistry*, *2020*, 1–8. https://doi.org/10.1155/2020/9201457

Wildermuth, S. R., Young, E. E., & 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

Wintersohle, C., Kracke, I., Ignatzy, L. M., Etzbach, L., & Schweiggert-Weisz, U. (2023). Physicochemical and chemical properties of mung bean protein isolate affected by the isolation procedure. *Current Research in Food Science*, *7*, 100582. https://doi.org/10.1016/j.crfs.2023.100582

Wong, J. C. J., Khoiroh, I., Gan, S., & Croft, A. K. (2025). Deep eutectic solvents for protein extraction: Mechanisms, performance, and green metrics. *Journal of Molecular Liquids*, *438*, 128636. https://doi.org/10.1016/j.molliq.2025.128636

Wu, G. (2016). Dietary protein intake and human health. *Food & Function*, *7*(3), 1251–1265. https://doi.org/10.1039/C5FO01530H

Wu, G., Bazer, F. W., Davis, T. A., Kim, S. W., Li, P., Marc Rhoads, J., Carey Satterfield, M., Smith, S. B., Spencer, T. E., & Yin, Y. (2009). Arginine metabolism and nutrition in growth, health and disease. *Amino Acids*, *37*(1), 153–168. https://doi.org/10.1007/s00726-008-0210-y

Yang, J., Kornet, R., Ntone, E., Meijers, M. G. J., Van Den Hoek, I. A. F., Sagis, L. M. C., Venema, P., Meinders, M. B. J., Berton-Carabin, C. C., Nikiforidis, C. V., & Hinderink, E. B. A. (2024). Plant protein aggregates induced by extraction and fractionation processes: Impact on techno-functional properties. *Food Hydrocolloids*, *155*, 110223. https://doi.org/10.1016/j.foodhyd.2024.110223

Yao, S., Li, W., Martin, G. J. O., & Ashokkumar, M. (2023). An investigation into the mechanism of alkaline extraction-isoelectric point precipitation (AE-IEP) of high-thiol plant proteins. *Applied Sciences*, *13*(11), 6469. https://doi.org/10.3390/app13116469

Zaky, A. A., Hussein, A. S., Mostafa, S., & Abd El-Aty, A. M. (2022). Impact of sunflower meal protein isolate supplementation on pasta quality. *Separations*, *9*(12), 429. https://doi.org/10.3390/separations9120429

Zardo, I., De Espíndola Sobczyk, A., Marczak, L. D. F., & Sarkis, J. (2019). Optimization of ultrasound assisted extraction of phenolic compounds from sunflower seed cake using response surface methodology. *Waste and Biomass Valorization*, *10*(1), 33–44. https://doi.org/10.1007/s12649-017-0038-3

Zhang, X., Liu, Z., Ma, X., Zheng, Y., Hu, H., Jiao, B., McClements, D. J., Wang, Q., & Shi, A. (2025). Interfacial and foaming properties of plant and microbial proteins: Comparison of structure-function behavior of different proteins. *Food Chemistry*, *463*, 141431. https://doi.org/10.1016/j.foodchem.2024.141431

Zhang, Y., Roman, L., Kirkensgaard, J. J. K., & Corredig, M. (2025). Effect of salt extraction on composition, structure, and thermal properties of pea protein. *Food Chemistry*, *486*, 144650. https://doi.org/10.1016/j.foodchem.2025.144650

Zhang, Y., Sharan, S., Rinnan, Å., & Orlien, V. (2021). Survey on methods for investigating protein functionality and related molecular characteristics. *Foods*, *10*(11), 2848. https://doi.org/10.3390/foods10112848

Appendix

Appendix 1. Protein solubility (mg/mL) at different pH for sunflower meal (SFM) and sunflower protein isolates (SFPI) extracted with four methods.

Sample	Protein solubility (mg/mL)			
	pH 3	**pH 5**	**pH 7**	**pH 9**
SFM	8.85 ± 0.23 ^{A}	8.88 ± 0.37 ^{A}	28.58 ± 3.13 ^{B}	60.06 ± 5.64 ^{B}
DV-SFPI	8.38 ± 0.32 ^{A}	8.66 ± 0.15 ^{A}	28.52 ± 1.71 ^{B}	45.98 ± 3.46 ^{B}
MV-SFPI	8.32 ± 0.29 ^{A}	8.36 ± 0.30 ^{A}	47.19 ± 2.37 ^{A}	51.88 ± 3.13 ^{B}
DES-SFPI	8.39 ± 0.14 ^{A}	8.92 ± 0.11 ^{A}	24.83 ± 1.03 ^{B}	64.09 ± 3.98 ^{B}
ALK-SFPI	8.69 ± 0.27 ^{A}	6.83 ± 0.28 ^{B}	30.43 ± 0.89 ^{B}	87.67 ± 3.11 ^{A}

$^{A-B}$ Superscripts denote a significant difference between samples, $P < 0.05$ using Tukey's test.

Appendix 2. Water and oil holding capacity (g/ g protein) for sunflower meal (SFM) and sunflower protein isolates (SFPI).

Sample	Water holding capacity (g water/g protein)	Oil holding capacity (g oil/g protein)
SFM	0.85 ± 0.03 ^{A}	0.77 ± 0.003 ^{E}
DV-SFPI	0.40 ± 0.04 ^{BC}	1.48 ± 0.01 ^{B}
MV-SFPI	0.30 ± 0.03 ^{C}	1.11 ± 0.004 ^{D}
DES-SFPI	0.52 ± 0.03 ^{B}	1.62 ± 0.01 ^{A}
ALK-SFPI	0.78 ± 0.01 ^{A}	1.30 ± 0.01 ^{C}

$^{A-E}$ Superscripts denote a significant difference between samples, $P < 0.05$ using Tukey's test.

Appendix 3. Foaming capacity and stability (%) at different pH for sunflower meal (SFM) and sunflower protein isolates (SFPI).

Sample	Foaming capacity (%)				Foaming stability (%)			
	pH 3	**pH 5**	**pH 7**	**pH 9**	**pH 3**	**pH 5**	**pH 7**	**pH 9**
SFM	13.33 ± 0.83 ^{B}	7.50 ± 0.00 ^{D}	18.33 ± 2.21 ^{C}	16.67 ± 1.67 ^{B}	44.44 ± 8.01 ^{B}	61.11 ± 5.56 ^{A}	82.28 ± 2.35 ^{A}	79.17 ± 6.37 ^{A}
DV-SFPI	58.33 ± 7.95 ^{A}	77.50 ± 9.46 ^{A}	45.83 ± 0.83 ^{A}	29.17 ± 3.63 ^{AB}	82.21 ± 3.29 ^{A}	29.43 ± 1.65 ^{B}	85.48 ± 1.72 ^{A}	82.28 ± 2.35 ^{A}
MV-SFPI	51.67 ± 0.83 ^{A}	56.66 ± 2.21 ^{AB}	25.00 ± 1.44 ^{BC}	27.50 ± 2.50 ^{AB}	79.05 ± 1.46 ^{A}	28.14 ± 2.62 ^{B}	83.57 ± 2.71 ^{A}	84.87 ± 2.89 ^{A}
DES-SFPI	55.00 ± 1.44 ^{A}	48.33 ± 6.67 ^{BC}	40.00 ± 0.00 ^{A}	24.17 ± 3.01 ^{AB}	75.86 ± 2.57 ^{A}	34.84 ± 7.58 ^{B}	87.50 ± 0.00 ^{A}	86.57 ± 1.67 ^{A}
ALK-SFPI	28.33 ± 2.21 ^{B}	25.83 ± 0.83 ^{CD}	29.16 ± 3.33 ^{B}	30.83 ± 2.21 ^{A}	67.62 ± 2.01 ^{A}	57.87 ± 4.08 ^{A}	86.04 ± 1.43 ^{A}	76.08 ± 3.05 ^{A}

$^{A-D}$ Superscripts denote a significant difference between samples, $P < 0.05$ using Tukey's test.

Appendix 4. Emulsification activity and stability index at different pH for sunflower meal (SFM) and sunflower protein isolates (SFPI).

Sample	Activity index (m^2/g)				Stability index (min)			
	pH 3	**pH 5**	**pH 7**	**pH 9**	**pH 3**	**pH 5**	**pH 7**	**pH 9**
SFM	1.59 ± 0.19 ^{C}	5.18 ± 0.03 ^{B}	10.55 ± 0.29 ^{A}	11.42 ± 0.50 ^{A}	23.63 ± 1.00 ^{A}	10.51 ± 0.09 ^{A}	10.29 ± 0.10 ^{A}	11.70 ± 0.98 ^{A}
DV-SFPI	9.11 ± 0.33 ^{A}	5.46 ± 0.26 ^{B}	5.65 ± 2.02 ^{AB}	9.76 ± 0.74 ^{A}	13.46 ± 2.15 ^{B}	10.42 ± 0.05 ^{A}	11.00 ± 0.43 ^{A}	11.67 ± 0.70 ^{A}
MV-SFPI	8.49 ± 0.54 ^{AB}	7.22 ± 0.08 ^{A}	3.72 ± 0.93 ^{B}	10.85 ± 0.96 ^{A}	11.20 ± 0.37 ^{B}	10.36 ± 0.02 ^{A}	10.64 ± 0.17 ^{A}	10.35 ± 0.10 ^{A}
DES-SFPI	7.46 ± 0.09 ^{AB}	6.16 ± 0.28 ^{B}	4.21 ± 1.00 ^{B}	10.47 ± 0.29 ^{A}	10.49 ± 0.18 ^{B}	10.43 ± 0.12 ^{A}	10.23 ± 0.04 ^{A}	10.57 ± 0.12 ^{A}
ALK-SFPI	7.16 ± 0.38 ^{B}	5.54 ± 0.20 ^{B}	9.47 ± 0.52 ^{A}	11.74 ± 0.37 ^{A}	10.45 ± 0.13 ^{B}	10.22 ± 0.03 ^{A}	11.22 ± 0.34 ^{A}	10.57 ± 0.03 ^{A}

$^{A-C}$ Superscripts denote a significant difference between samples, $P < 0.05$ using Tukey's test.

Appendix 5. Amino acid scoring reference pattern for children of preschool age (2–5 years), proposed by FAO/WHO.

Amino acid	mg/g crude protein
Histidine (His)	19
Isoleucine (Ile)	28
Leucine (Leu)	66
Lysine (Lys)	58
Methionine + Cystine (Met + Cys)	25
Phenylalanine + Tyrosine (Phe + Tyr)	63
Threonine (Thr)	34
Tryptophan (Trp)	11
Valine (Val)	35