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Isolation of Protein from Sweet-Lime seed using Sustainable High-Intensity Ultrasound Technique-Physicochemical and Functional characteristics

Kimeera Ambati[1*], A.M. Beulah[2], G. Sireesha[3] and K. Kumaraguru[4]

[1,2,3]Department of Home Science, Sri Padmavati Mahila Visvavidyalayam, Tirupati - 517502 (India)
[4]Department of Food Processing Technology, PSG College of Arts and Science, Coimbatore - 641014 (India)
*sireeshaguttapalam@gmail.com

Abstract

The study explores the isolation of protein from sweet-lime seed using a sustainable high-intensity ultrasound technique and examines its physicochemical and functional characteristics. The ultrasound-assisted extraction process was optimized for maximum yield and efficiency, while minimizing environmental impact compared to conventional methods. The extracted protein isolate was characterized for its physicochemical properties, including Bulk density, pH and water activity. Functional properties like water holding capacity, foaming ability, and emulsifying were analysed. Results showed that ultrasound-assisted extraction significantly enhanced protein yield and quality, with improvements in functional attributes that are critical for applications in food formulations. The study highlights the potential of sweet-lime seed protein as a sustainable and functional ingredient, contributing to the broader goal of utilizing agro-industrial byproducts for food innovation.

Key words : Sweet-lime seed protein, Ultrasound-assisted extraction, Sustainable technology, Physicochemical characteristics, Functional properties.

High-intensity ultrasound (HIU) is a transformative technology that has been increasingly applied in protein processing. Its non-thermal, mechanical energy is non-invasive and environmentally friendly, making it an ideal method for modifying the functional properties of proteins. Studies have shown that HIU can enhance protein solubility, reduce particle size, and alter structural properties, which can improve the efficacy of protein-based food products[8]. This process, which involves the application of ultrasound energy, can lead to changes in protein structure, resulting in improved solubility, emulsification, and gelation

[1,2,3]Assistant Professors, [4]Assistant Professor,

properties. Such modifications are beneficial in various industries, particularly in food science, where HIU is used to enhance the functionality of food proteins, making them more suitable for different applications and products[9]. It involves the application of ultrasound at frequencies typically around 20 kHz, which can alter the structural properties and physicochemical properties of proteins due to hydrodynamic shearing and heating and alterations in secondary structures like α-helices and β-sheets. This technology is considered ecological, innovative, and sustainable[10].

The disposal of sweet-lime seeds and other fruit by-products poses significant environmental challenges. These wastes contribute to greenhouse gas emissions and can lead to soil degradation and water contamination. However, there are sustainable ways to manage these by-products. Composting and biochar production are viable strategies that can mitigate the environmental impact by transforming waste into valuable resources for soil amendment. Furthermore, investigating different use for sweet lime seeds, such as in cooking or as a source of nutrients, may help cut waste and advance a circular economy. The juice processing sector receives around 50 and 60 percent of the total production of sweet lime fruits. In particular, a significant amount of it (segment membranes, peels, and seeds) is produced during the juice-making process and can account for 50–70% of the fruit's weight. Up to 40–55% of the fruit is made up of the skin, and up to 10% is made up of the seeds. Isolating the protein from sweet lime seed flour and examining the effects of high-intensity ultrasound delivered by an ultrasonic bath on technological-functional and physicochemical qualities are the primary goals of the current study.

The juicy lime seeds were gathered at a juice extraction store in Tirupati's local marketplaces. In order to remove the foreign material and drain the extra water in a plastic sieve, the delicious lime seeds were physically separated from running water. The seeds were used to make protein isolates from sweet lime seeds (SS). Hi-Media Laboratories Pvt. Ltd. provided the analytical grade chemicals and reagents.

Preparation of the Defatted Sweet-lime Seed Flour (DSSF) :

The clean seeds were then put in a plastic mesh-lined tray and dried for 72 hours at 30 °C and 3.0 m s^{-1} in a modified convection drying oven. The seeds' pericarp was then manually scraped, and the resulting coarse powder—known as sweet-lime seed flour—was then blended. By immersing 100g of the sweet-lime seed flour (SSF) in 1l of ethylic ether for an hour and replacing the used solvent with a new one, the SSF is defatted. After 12 hours of desolventization in a fume extraction hood, the material is ground into defatted Sweet-lime seed flour (DSSF) in a Cyclotec 1093 mill and kept for further analysis[2,3].

Preparation of Sweet-lime Seed Protein Isolate (SSPI) and Application of Ultrasound Treatment :

First, lots of 50 g of DSSF are used to produce SSPI by alkaline extraction and isoelectric precipitation, where the DSSF protein solubility is specified in the pH range

of 2 to 12. The DSSF and distilled water are combined at a 1:20 ratio, and 1.0 M NaOH is used to bring the mixture's pH down to the maximum protein extraction. After 30 minutes of stirring at 25 °C, the slurry is centrifuged at 4500 rpm. The slurry will be agitated for 20 minutes at 25°C after the pH of the supernatant is brought down to the pH of the minimal protein extraction (isoelectric point) using 1.0 M HCl. Centrifugation is used to separate the precipitate for 20 minutes at 25 °C and 4500 rpm. Centrifugation is used to separate the precipitate for 20 minutes at 25 °C and 4500 rpm. The protein precipitate is then re-suspended in distilled water at a 1:10 ratio and treated with 0.1 N NaOH to bring its pH down to 7. After that, a protein suspension was subjected to 15 minutes of ultrasonic therapy. A Branson ultrasonic bath model MYH-3510 (42 kHz, 130 W; tank capacity of 5 L; interior dimensions of 290 × 150 × 150 mm) with an acoustic density of 0.026 W/cm^3 and 25 °C is used to apply the ultrasound treatment. To receive the ultrasonic treatment, the beaker with the protein solution is positioned in the middle of the bath. A control therapy that does not involve the use of ultrasonography is also prepared. Lastly, the protein suspensions that have undergone control and ultrasound treatment will be lyophilized in a Free Zone 10 L model apparatus and kept for subsequent usage in hermetically sealed glass bottles at 25°C. [1,6,7].

Chemical Composition :

Standard AOAC techniques were used to analyze the chemical composition of SSPI, including its moisture, ash, protein, and lipid concentrations[4].

Physicochemical characteristics :

Bulk density :

The bulk density of sweet-lime seed protein isolates (SSPIs) was determined by the volume-displacement method.

pH :

The pH of the sweet-lime seed protein isolate (SSPI) suspension was measured using a digital pH meter (Metrohm AG, Switzerland).

Water activity :

The water activity (aw) of sweet-lime seed protein isolates (SSPIs) was measured using a water activity meter (Aqualab Series 4TE, Decagon Devices, Inc., USA).

Color analysis :

The color analysis of sweet-lime seed protein isolates (SSPIs) was conducted using a colorimeter (D-25, Hunter Associated Laboratory, USA).

Functional Characteristics :

Water holding capacity :

The water-holding capacity (WHC) of sweet-lime seed protein isolates (SSPIs) was assessed using the Ozyurt *et al.*[17] method.

Oil-holding capacity :

The oil-holding capacity (OHC) of sweet-lime seed protein isolates (SSPIs) was evaluated using the Ozyurt *et al.*[17] method, which determines the ability of SSPIs to retain oil.

Emulsifying capacity :

The method described by Ozyurt *et*

al.[17] was used for calculating the emulsifying capacity (EC) of SSPIs.

Foaming Properties :

The method described by Lawhon *et al.*[15] for examining the foaming capacity (FC) and foam stability (FS) of SSPIs was used.

Statistical analysis :

At $p<0.05$, the difference between the mean values was deemed significant. SPSS 21.0 was used to analyze the data (SPSS Inc. Chicago, USA).

Chemical composition :

The analysis of the chemical composition of the three samples Defatted Sweetlime Seed Flour (DSSF), Sweetlime Seed Protein Isolate (SSPI), Ultrasound assisted Sweetlime Seed Protein Isolate (USSPI) reveals a distinct variation in their constituent elements. The data, as presented in Table-1, indicates that moisture, protein, ash, and lipid contents vary significantly among the samples, with a p-value less than 0.05, denoting statistical significance. Notably, USSPI exhibits the highest protein content at 94.70%, surpassing SSPI, which contains 92.62% protein, and DSSF, which has 89.27%. These differences in protein levels could have implications for the sample applications and could influence further research or usage decisions based on the specific requirements of protein content.

The data presented in Table-1 reveals a comparative analysis of the moisture, ash, and lipid content across three distinct samples. DSSF exhibits the highest moisture content at 4.52%, with SSPI and USSPI following closely at 4.28% and 4.07%, respectively. In terms of ash content, USSPI registers the highest value at 0.67%, marginally surpassing SSPI 0.66%, while DSSF has the lowest at 0.60%, indicating a significant difference with a p-value less than 0.05. Additionally, the lipid content shows a descending trend from DSSF with the highest at 2.09%, to SSPI at 1.76%, and finally USSPI at 1.56%. These variations in composition could be indicative of differing processing methods or source variations, and they underscore the importance of detailed compositional analysis in quality control and product development.

Table-1. Chemical composition of untreated and treated sweet lime seed protein isolates

Sample	Chemical composition			
	Moisture (%)	Proteins (%)	Ash (%)	Lipids (%)
Hydrolysates				
DSSF	4.52 ± 0.08^{b}	89.27 ± 0.81^{c}	0.60 ± 0.05^{c}	2.09 ± 0.02^{b}
SSPI	4.28 ± 0.04^{c}	92.62 ± 0.07^{b}	0.66 ± 0.03^{a}	1.76 ± 0.05^{c}
USSPI	4.07 ± 0.05^{d}	94.70 ± 0.12^{a}	0.67 ± 0.02^{a}	1.56 ± 0.02^{d}

All data were means of triplicates. Values with the same superscripts in a column did not differ significantly ($p < 0.05$) by DMRT. DSSF- Defatted Sweetlime Seed Flour, SSPI-Sweetlime Seed Protein Isolate, USSPI- Ultrasound treated Sweetlime Seed Protein Isolate

Physicochemical properties :

Bulk density :

Bulk density is indeed a critical parameter in the food industry, serving as a key indicator of sample mass, handling requirements, and the suitability of packaging materials for storage and transport. It reflects the product's behavior and is affected by various factors such as the method of preparation, drying process, particle size, and moisture content content[13]. The significant differences in bulk densities observed in samples DSSF, SSPI and USSPI, as reportedly in Table-2 had varying bulk densities of 378.3, 330.2 and 310.6 kg/m^3 respectively, underscore the variability that can arise from these factors. The structure of protein molecules, their hydrophobicity, solubility, and hydrodynamic properties are known to influence the bulk density of protein isolates[16]. This is particularly important in the context of weaning food formulations, where a lower bulk density is preferred to ensure the food is light and digestible for infants. Understanding these properties can lead to better design and optimization of food products to meet specific nutritional and functional requirements.

pH :

The pH levels of sweet-lime seed flour, as indicated by the data in table-2, are crucial for assessing its quality and suitability for various applications. The significant difference in pH values between DSSF (8.6), SSPI (8.52), and USSPI (8.51) suggests variability that could impact the flour's functional properties, such as foaming and emulsification, which are essential for food science and technology. These properties, along with water activity, are key factors in determining the shelf life of the flour, as they affect microbial growth and product stability. Therefore, understanding and controlling the pH levels is vital for optimizing the use of sweet-lime seed flour in food products, ensuring both safety and quality. The statistical significance ($p<0.05$) of the differences further underscores the need for precise pH control during processing and storage.

Water activity :

Water activity is a critical factor in determining the shelf life and safety of food products. It measures the free water available for microbial growth, which is why lower water activity can inhibit the proliferation of bacteria, yeast, and molds, thereby extending the shelf life of food. Primo-Martin *et al*.[18] highlights the protective role of lower water activity against microbial contamination and biochemical degradation. The significant differences in water activity levels DSSF, SSPI and USSPI had varying water activities of 0.25, 0.233 and 0.215 respectively, as indicated by the p-value (<0.05), suggest that even small variations can have a substantial impact on food preservation. Furthermore, the strategy mentioned by Suriya *et al*.[20] to prevent moisture migration by introducing an edible layer is an innovative approach to modify water activity and enhance the stability of food systems. This technique can be particularly beneficial in multi-component foods where different ingredients have varying water activities, helping to maintain the desired quality and extend the product's shelf life.

Color :

The study of color parameters in food products, such as sweet-lime seed flour, is crucial

for understanding consumer acceptance. The L*, a*, and b* values provide a quantitative measure of color, which can be directly correlated with the product's appearance and perceived quality. USSPI had the highest L^* value (95.42%), followed by SSPI (95.13%). The lowest L^* value was noted for DSSF (94.09%). The lightness value (L*) indicates a high degree of brightness in sweet-lime seed flour, surpassing that of commonly known proteins like Alaska pollock (76.0%) (Sathivel and Bechtel 2006), and saithe (41.2%) (Shaviklo et al. 2012), suggesting a visually lighter flour. The a^* values of DSSF, SSPI and USSPI were up to -0.405%, -0.385% and -0.405%, respectively, with a significant difference ($p<0.05$). These values are comparable to those of kidney bean and pea proteins[19]. The b* values, representing the yellow-blue chromaticity, show significant variation, which could impact the final product's color appeal. Data were noted in b^* values among SSPIs, and the values ranged between 4.27% and 6.08%. The chroma and hue angle further describe the intensity and type of color the human eye perceives, with the sweet-lime seed flour displaying a high chroma, indicative of a vibrant color. Chromaticity was calculated as chroma (C^*), which denotes the fullness of color. The protein isolates exhibited maximum chromaticity (6.08). The hue angle denotes the sensitivity of color. From the findings, the hue angle of all SSPIs ranged between 95.42° and 95.13°. In addition, the color parameters of SSPIs can be influenced by various factors including the isolation process, temperature, ice-cold washing, and drying process.

These findings highlight the potential of sweet-lime seed flour as an ingredient in food products where color is a determining factor for consumer preference. Moreover, the influence of processing conditions on these color parameters underscores the importance of controlled manufacturing processes to

Table-2. Physico-chemical characteristic of untreated and treated sweet lime seed protein isolates

Parameters	Samples		
	DSSF	SSPI	USSPI
Bulk density (kg/m^3)	378.3 ± 3.21^c	330.2 ± 5.12^b	310.6 ± 7.52^a
pH	8.60 ± 0.10^a	8.52 ± 0.05^b	8.51 ± 0.07^b
Water activity	0.250 ± 0.05^a	0.233 ± 0.02^b	0.215 ± 0.06^c
L^*	94.09 ± 0.18^b	95.13 ± 0.74^a	95.42 ± 0.66^a
a^*	-0.405± 0.13^b	-0.385 ± 0.11^c	-0.405 ± 0.03^b
b^*	6.06 ± 0.19^b	6.81 ± 0.12^a	4.27± 0.51^c
Chromaticity	6.08 ± 0. 12^b	5.54 ± 0.12^c	4.31 ± 0.71^d
Hue Angle (°)	94.09 ± 0.18^b	95.13 ± 0.74^a	95.42 ± 0.66^a

All data were means of triplicates. Values with the same superscripts in a column did not differ significantly ($p < 0.05$) by DMRT. DSSF- Defatted Sweetlime Seed Flour, SSPI-Sweetlime Seed Protein Isolate, USSPI- Ultrasound treated Sweetlime Seed Protein Isolate

maintain consistent quality in food products. The detailed analysis of these color attributes can guide food technologists in optimizing the visual aspects of new and existing food items, ensuring they meet consumer expectations and industry standards.

Functional characteristics :

Water holding capacity :

The Water Holding Capacity (WHC) of different protein isolates is a critical factor in food science, as it affects the sensory properties and processing behavior of food products. The data presented in Table-3 indicates that the Sweetlime samples have a higher WHC ranged between 5.90 and 5.17 mL/g than commonly used proteins such as tilapia (2.63 mL/g), soy (4.05 mL/g), and wheat (3.67 mL/g), which suggests that these isolates may offer improved functional properties in food systems[5,13]. The significant variation in WHC among the SLPI samples could be attributed to differences in their composition or structure, with USSPI exhibiting the highest capacity. This superior WHC can enhance the product's mouthfeel, flavor retention, and texture, which are essential qualities for consumer acceptance and satisfaction. Furthermore, the carbohydrate content's influence on WHC underscores the importance of considering the interplay between different food components to optimize the quality of food products.

Oil holding capacity :

The Oil Holding Capacity (OHC) of food materials is a critical parameter in food science, as it influences the texture, mouthfeel, and stability of food products. The data presented in Table-3 highlights the superior OHC of the samples, DSSF, SSPI and USSPI had varying OHC values of 6.20, 6.43 and 6.51 mL/g, respectively, with a significant difference ($p<0.05$) which surpasses that of commonly used proteins such as quinoa (1.88 mL/g), tilapia (3.38 mL/g), and soya (2.81 mL/g)[12]. This suggests that the samples, possibly due to their unique protein composition or structural properties, have a higher affinity for oil absorption. Factors like protein type, hydrolysis level, and interaction with different oils can significantly impact OHC, as noted by Cumby *et al.*[11]. The enhanced water and oil absorption properties of these samples, referred to as SSPIs, can be leveraged to improve the sensory and functional qualities of various food items, making them more appealing to consumers. This could lead to innovations in food processing and product development, particularly in enhancing the palatability and nutritional value of processed foods.

Emulsifying capacity :

Emulsifying capacity (EC) is a critical parameter in food science, reflecting a substance's ability to stabilize emulsions, which are mixtures of oil and water that typically do not mix well. The EC is particularly important in the context of proteins, which can act as emulsifiers, aiding in the dispersion of oil droplets in water. The isolation procedure, along with the inherent physicochemical properties of the proteins, such as molecular size, structure, pH, hydrophobicity, solubility, and surface charge, significantly affect their emulsifying properties. The data presented indicates that the EC of the samples tested surpasses that of common proteins like soy and marama, and is on par with tilapia protein isolates, known for their high solubility and

emulsifying abilities. The EC of sample 1, sample 2 and sample 3 was up to 83.3%, 85.5% and 88.3%, respectively (Table 3), with a significant difference ($p<0.05$). These values are higher than those of soy protein (52.5%) and marama protein (53.4%)[14], whereas they are comparable to those of tilapia protein (83.7%)[13]. The SSPI has the promising applications in food products where emulsification is desired, potentially offering a superior alternative to traditional protein sources.

Foaming properties :

The study of protein foaming properties, such as foam capacity (FC) and foam stability (FS), is crucial in the development of food products where texture and consistency are key[6]. The research cited indicates that the FC of soybean protein isolate (SBPI) samples varies with protein concentration, suggesting a direct correlation between the two. The FC of DSSF, SSPI and USSPI was up to 15.2%, 22.2% and 36.7% respectively (Table-3), with a significant difference ($p<0.05$). From the findings, the FC of protein isolates was greatly improved with the increase in protein concentration. In addition, the FC values are lower than those of quinoa protein (58.37%)[12].

While the FC for SLPI samples shows a marked increase with higher protein concentrations, it still falls short when compared to quinoa protein, highlighting the diversity in foaming properties among different protein sources. Furthermore, the FS of all SLPIs ranged between 6.2% and 18.7%, 7.3% and 19.8%, and 9.2% and 19.8% for foam intervals at 15, 20, and 30 min, respectively, with a significant difference ($p<0.05$). Foam formation of proteins can be regulated by three major factors: transportation, penetration, and

Table-3. Functional characteristic and particle size of untreated and treated sweet lime seed protein isolates.

Parameter	SAMPLES		
	S1	S2	S3
WHC (mL/g)	5.17 ± 0.02^{c}	5.60 ± 0.08^{b}	5.90 ± 0.04^{a}
OHC (mL/g)	6.20 ± 0.12^{c}	6.43 ± 0.04^{b}	6.51 ± 0.06^{a}
EC (%)	83.3 ± 0.12^{c}	85.5 ± 0.05^{b}	88.3 ± 0.10^{a}
FC (%)	15.2 ± 0.09^{c}	22.2 ± 0.05^{b}	36.7 ± 0.02^{a}
FS at 15 min (%)	6.2 ± 0.09^{c}	15.2 ± 0.03^{b}	18.7 ± 0.10^{a}
FS at 20 min (%)	7.3 ± 1.08^{bc}	15.3 ± 0.02^{b}	19.2 ± 0.12^{a}
FS at 30 min (%)	9.2 ± 0.10^{d}	16.5 ± 0.10^{b}	19.8 ± 0.03^{a}
Emulsifying capacity (%)	13.2 ± 0.02^{d}	36.5 ± 0.07^{b}	59.8 ± 0.05^{a}
Emulsifying stability (%)	11.2 ± 0.10^{d}	22.5 ± 0.13^{b}	49.8 ± 0.13^{a}

All data were means of triplicates. Values with the same superscripts in a column did not differ significantly ($p < 0.05$) by DMRT. DSSF- Defatted Sweetlime Seed Flour, SSPI-Sweetlime Seed Protein Isolate, USSPI- Ultrasound treated Sweetlime Seed Protein Isolate

reorganization of molecules at the air/water interface[21]. These insights are invaluable for food scientists aiming to tailor the textural properties of food products to meet specific consumer preferences and requirements. In addition, these processes are influenced by numerous factors, such as protein solubility, equilibrium between the rigidity and flexibility of proteins, pH, hydrophobicity, temperature, and ionic strength[19].

The study demonstrated that the application of ultrasound technology (USSPI) significantly enhances the functional and physicochemical properties of Sweetlime Seed Protein Isolate (SSPI) compared to untreated Defatted Sweetlime Seed Flour (DSSF) and standard SSPI. The chemical composition analysis reveals that USSPI exhibits the highest protein content, lower moisture and lipid levels, and improved ash content, indicating its superior quality for protein-rich applications. This aligns with the observed lower bulk density, which is crucial for applications like weaning foods, where lower density ensures light and digestible properties.

Physicochemical properties such as pH, water activity, and color parameters further emphasize the significance of ultrasound processing. USSPI demonstrated a pH level conducive to maintaining functional properties, a lower water activity for better shelf stability, and color parameters that make it visually more appealing for food products. Its enhanced emulsifying capacity and superior water and oil holding capacities suggest that USSPI may perform better in food formulations where these functional properties are critical, such as emulsified products, bakery goods, and processed foods.

Conflict of interest

There is no Conflict of Interest

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