REVIEW ARTICLE

Ultrasound as a Technique to Extract Plant Proteins: Effects, Yields and Modifications

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Abstract

Plant-based proteins have been promoted in research for various reasons. First, more people choose to live sustainably with environmental concerns. Moreover, adequate proteins are required for the new challenge in an expanding world population. Furthermore, from an industrial perspective, extracting nutrients from cheaper sources with better value in both nutrients and markets is in demand. Proteins from traditional sources such as soy and wheat, seeing potential value in other protein-rich legumes, seeds, or even leaves, have been considered as sources over the past 20 years in approximation. The extractions can be done traditionally by thermal or alkali methods, while other novel techniques, including ultrasonication, fit the need to improve extraction efficiency and better functional properties and less energy-intensive. Ultrasonic-assisted extraction (UAE), mostly applied in alkaline conditions, and aqueous extraction with variations, have improved yield and better functionality in most plants, compared to the traditional alkaline method. In practical use, multiple established methods are applied together to accomplish the best results, apart from limitations. The review aims to examine the data from these results and to provide general evidence of availability, meanwhile mentioning some definite challenges in real manufacturing environments. Regarding its better applications to manufacturers, possible guidelines in practice are presented.

Keywords: UAE, alkaline, extraction, yield, protein, functional properties, antioxidant.

Abbreviation	Definition
AE	(Traditional) Alkaline Extraction
EC/ES	Emulsion Capacity/ Stability
EAI/ESI	Emulsifying Activity/ Stability Index
FC/FS	Foaming Capacity/ Stability
FAC/WAC	Fat/ Water Absorption Capacity
FE	Foaming Expansion
UAE	Ultrasonic-Assisted Extraction
UE	Ultrasound and Enzymatic Extraction
UUAAIP	Ultrasound-Ultrafiltration-Assisted Alkaline Isoelectric Precipitation
PH	Protein Hydrolysates
SHF	Free Sulfhydryl
SS	Disulfide Bond
TPC	Total Phenol Content
WHC/OHC	Water-/ Oil-Holding Capacity
WBC/OBC	Water-/ Oil-Binding Capacity
L/S	Liquid-Solid Ratio
SE-HPLC	Size-Exclusion High-Performance Liquid Chromatography

Submitted: 16 June 2025
Accepted: 28 June 2025
Published: 29 June 2025

Vol. 2, **No.** 2, 2025.
10.62762/ASFP.2025.102130

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Citation
Zhan, Y. (2025). Ultrasound as a Technique to Extract Plant Proteins: Effects, Yields and Modifications. *Agricultural Science and Food Processing*, 2(2), 89–105.

1 Introduction

UAE (ultrasound-assisted extraction), one of the novel methods in greener commercial production, is claimed to be more efficient and effective in extractions [1–4]. Ultrasonic waves are known as pressure waves with frequencies typically exceeding 18 kHz [5], which is above the range perceived by the human ear and carry certain energy. This characteristic enables its use in extractions involving more complex structures. And in past two decades, it is applied in by-products protein recovery, protein extraction in plant sources, and improving efficiency combined with other techniques, together with energy saving strategies (Figure 1).

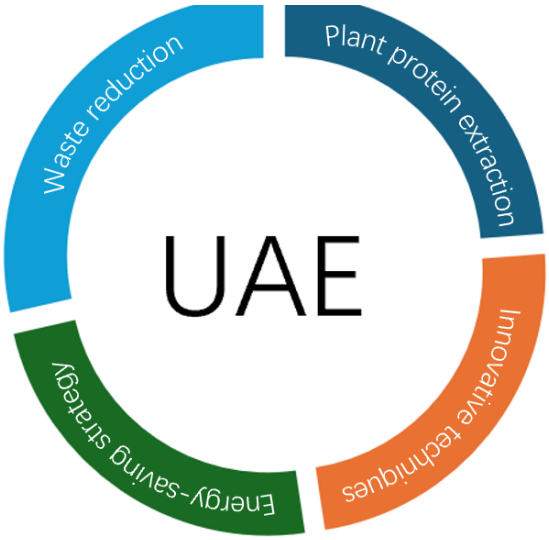

Figure 1. General description on current UAE trends.

Ultrasound can be classified by its intensity and frequency. The division based on intensities measured in amplitude may use the following rule in classification - 5, 10, and 15 μm-pp, in low, medium and high intensity, respectively [2]. The 'μm-pp' refers to peak-to-peak amplitude in μm. Moreover, pulsed ultrasound has been mentioned in Das et al. [5] and Li et al. [6], measured with either pulse (s) directly or DC (%). Based on frequency or power intensity, ultrasound can generally divide into either two — low frequency/high power (20-100 kHz and power 10–1000 W/cm^2) and high frequency/low power (5-10 MHz and power less than 1 W/cm^2) ultrasound [53] or three main classes — low (20–100 kHz), high (100–1000 kHz), and diagnostic ultrasonic (1–500 MHz) [7, 8]. High frequency/low power is applied in diagnostic analysis of food materials and various extraction processes in food industries, and the low frequency one with more energy tends to be used in clinical settings other than food [4, 7]. Additionally, the classification can be seen as in Figure 2 due to its applications under different conditions. It is mostly applied in improving nutrient value and extraction of protein as well as oil (as studied before), and some extracted protein may even apply in pharmaceutical needs due to its specific compounds or unique properties [21, 23, 26]. Though certain treatments led to oversonication may hinder its use, in most conditions, the effects are minor if properly controlled.

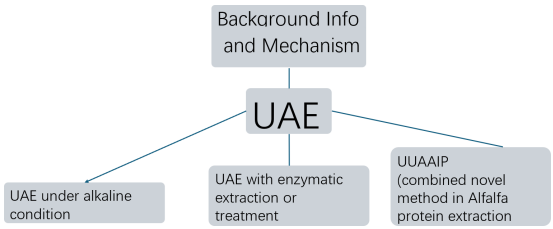

Figure 2. UAE general description. Based on the applications, there have been commercialized UAE under alkaline conditions, Ultrasound and Enzymatic extraction (UE) and UUAAIP. UAE in basic solution was cconcerered the most, and UE the developing one in preserving additional values in proteins, UUAAIP is only seen in Alfalfa leaf protein extraction.

The cavitation phenomenon, identified as a critical factor in increasing the yield of ultrasound, induces the explosion of hidden compounds in cells into the extraction medium [10, 11]. It is believed that the improved extraction efficiency results from the propagation of shock waves with pressure, causing microbubbles to form and grow in the liquid medium, with accumulation in sufficiently negative threshold pressure due to the alternating mechanical waves [12]. The gas nucleus, which appeared in the medium, can form cavitation bubbles (Figure 3). As the liquid temperature rises, vapour pressure increases with decreases in viscosity and tensile strength, which reduce the cavitation threshold, facilitating the formation and growth of bubbles [9]. Then the bubbles imploded, and harsh conditions in temperature (5000 K) and pressure (2000 atm), known as local physical effects, ignited after cavitation, alongside strong hydrodynamic turbulence and shear force [4]. Meanwhile, microjets are formed as the coalescence of bubbles, which can penetrate into surfaces nearby. During the process, another main effect, a diffusion effect [5], is supposed to appear. Great impact on properties like foaming is found, however was less mentioned. The diffusion of protein in the air-water interface, encouraging more air bubble entrapment, resulting in flexible structures [60]. The microstructure, as a key of the processing materials, changed regarding its impact. Therefore, reduction in

yield may be caused by the phenomenon [5, 23], since diffusivity is enhanced by the driving force in mass transfer, which also improves protein desorption [35].

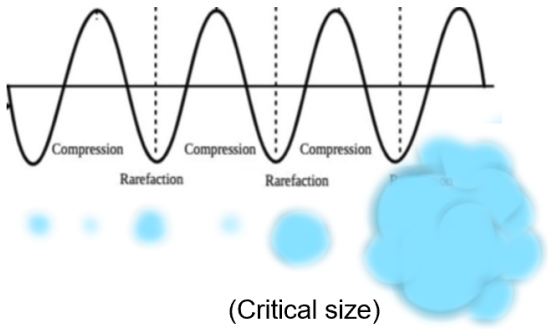

Figure 3. The growth of the bubbles and implosion thereafter under cavitation.

Cavitation and mechanical forces (vibration) may result in protein unfolding with either its following aggregation or dissociation of the aggregates [13–15, 17, 18] depending on the situation. The combination of those may further accelerate mass transfer, resulting release of protein and polyphenols from cell wall, aid by local turbulence, promoting probable destruction [36–39]. This also accelerates the effects of ultrasound on the chemical structure of proteins [16]. Hence, strong changes in protein function and particle sizes would take their place [15]. Solubility changes due to the particle sizes and aggregation size in proteins, whereas the formation of intermolecular disulfide bridges is responsible for the latter, which was caused by enhanced oxidation of cysteine as a result of hydroxyl-free radicals [16]. Oversonication may be seen for sensitive proteins due to the changes [28] regarding time, which would hinder the mass transfer effect due to the same effect in ultrasound.

Various UAE conditions have been conducted depends on the needs (Table 1). Commonly used in strong alkali conditions, it facilitates the release of proteins, and pH 5.5 for precipitation of solubilised proteins is suggested [19]; high pH (9-10) loosen the texture via disruption of hydrogen bonds, further causing the separation of hydrogen ions from the sulphate and carbolic groups [20]. Additionally, enzymes were used for hydrolysis and others to improve properties of the proteins and other compounds with better bioactivity preserved [21]. For instance, alkaline proteinase has been already used for improving solubility and digestibility in rice protein [22]; viscozyme L and alcalase have been used for higher total phenol compounds in protein extraction with less oxidation and higher antioxidant properties [21], and UUAAIP is seen in recent extraction for alfalfa leaf proteins [23, 24].

2 Legume Protein Extraction

The beans in the legume family, also known as pulses, are rich in valuable proteins. Soybeans were well studied compared to others in this family. UAE is applied to most of the pulses in either extraction or modification, with their yield and properties improved, allowing profound applications in future food processing. Figure 4 shows some plants proteins involved in the review.

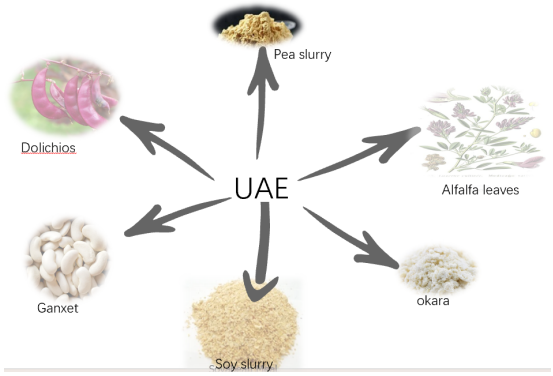

Figure 4. Certain protein sources from the legume family.

2.1 Soy protein extract and modification

Soy milk, which is high in demand in the current market [25] and has possible health benefits matched in nutritional plant-based beverages, has drawn research concerns. Earlier studies of soy-based systems investigating UAE showed improved extraction yields and enhanced functionality, such as the study in Fahmi et al. [9]. Moreover, soy meal and okara, a by-product during soybean oil extraction and a by-product during soymilk extraction, respectively, are rich in protein. Containing notable portions of protein (26.8-37.5% w/w), okara has caught great interest in recent studies, whose functional properties have seen their potential applications in beef burger and other definite formulations [2]. Regarding its scarce utilisation around 2022, the extraction is made to yield its protein with better properties. Das et al. [5] investigated the extraction from soy meal in a more recent study to give evidence for soy meal value.

2.1.1 Yield in soy meal, okara and soy milk with their modifications

Ultrasonication enhances the release of proteins from soybean cotyledon by utilising cavitation. The

Table 1. Frequency of UAE use under different conditions.

Classification	Article title	Year	Source
UAE under alkaline conditions (pH >7)	Effects of Temperature, Sonication Time, and Power Settings on Size Distribution and Extractability of Total Wheat Flour Proteins as Determined by Size-Exclusion High-Performance Liquid Chromatography	2000	https://doi.org/10.1094/CCHEM.2000.77.5.685
	Ultrasonic assisted alkali extraction of protein from defatted rice bran and properties of the protein concentrates.	2009	https://doi.org/10.1111/j.1365-2621.2009.02009.x
	Optimisation of ultrasonic-assisted protein extraction from brewer's spent grain	2010	https://doi.org/10.17221/178/2009-cjfs
	Characterization of the sesame (Sesamum indicum L.) global transcriptome using Illumina paired-end sequencing and development of EST-SSR markers.	2011	https://doi.org/10.1186/1471-2164-12-451
	Effect of Ultrasound Assisted Extraction upon the Protein Content and Rheological Properties of the Resultant Soymilk	2011	https://www.semanticscholar.org/paper/f86cfdcb523754d5ecc54fbb4396fea7a1f5141e
	Optimization of ultrasound-assisted extraction process of perilla seed meal proteins.	2012	https://doi.org/10.1007/s10068-012-0226-7
	Ultrasonic-assisted extraction of protein from rapeseed (Brassica napus L.) meal: Optimization of extraction conditions and structural characteristics of the protein	2017	https://www.proquest.com/scholarly-journals/ultrasonic-assisted-extraction-protein-rapeseed/docview/1914809023/se-2
	Characterization of functional properties of proteins from Ganxet beans (Phaseolus vulgaris L. var. Ganxet) isolated using an ultrasound-assisted methodology.	2018	https://doi.org/10.1016/j.lwt.2018.08.033
	Optimization of ultrasound assisted extraction of protein from sunflower meal and its physicochemical and functional properties.	2018	https://doi.org/10.1111/jfpe.12799
	Effects of divergent ultrasound pretreatment on the structure of watermelon seed protein and the antioxidant activity of its hydrolysates	2019	https://doi.org/10.1016/j.foodchem.2019.125165
	Reduction of the process time in the achieve of rice bran protein through ultrasound-assisted extraction and microwave-assisted extraction	2019	https://doi.org/10.1080/01496395.2019.1577449
	Sesame bran as an unexploited by-product: Effect of enzyme and ultrasound-assisted extraction on the recovery of protein and antioxidant compounds.	2019	https://doi.org/10.1016/j.foodchem.2019.01.077
	Conformational changes of soy proteins under high-intensity ultrasound and high-speed shearing treatments.	2019	https://doi.org/10.1021/acssuschemeng.8b05713
	Effects of ultrasound-assisted extraction on the structural, functional and antioxidant properties of Dolichos lablab L. Protein	2021	https://doi.org/10.1016/j.procbio.2020.11.027
	Modification of structural and functional characteristics of brewer's spent grain protein by ultrasound assisted extraction	2021	https://doi.org/10.1016/j.lwt.2020.110582
	Properties of protein isolates extracted by ultrasonication from soybean residue (okara)	2022	https://doi.org/10.1016/j.foodchem.2021.130837
	Ultrasonic extraction of soy protein isolate: Characterization and comparison with microwave and enzymatic extraction methods.	2023	https://doi.org/10.1111/1750-3841.16654
UAE under neutral pH	Intensified soy protein extraction by ultrasound	2017	https://doi.org/10.1016/j.cep.2016.09.003
Enzymatic- UAE	Ultrasound enhanced glucose release from corn in ethanol plants	2007	https://doi.org/10.1002/bit.21497
	An efficient ultrasound-assisted extraction method of pea protein and its effect on protein functional properties and biological activities.	2020	https://doi.org/10.1016/j.lwt.2020.109348
	Ultrasound-assisted alkaline proteinase extraction enhances the yield of pecan protein and modifies its functional properties.	2021	https://doi.org/10.1016/j.ultsonch.2021.105789
UUAAIP	Optimisation of steam blanching on enzymatic activity, color and protein degradation of alfalfa (Medicago sativa) to improve some quality characteristics of its edible protein.	2019	https://doi.org/10.1016/j.foodchem.2018.10.049
	Application of Ultrasound-Ultrafiltration-Assisted alkaline isoelectric precipitation (UUAAIP) technique for producing alfalfa protein isolate for human consumption: Optimization, comparison, physicochemical, and functional properties.	2020	https://doi.org/10.1016/j.foodres.2019.108907

conventional extraction (AE) of soybean proteins using various solvents is unpleasant and tends to have more pollution, which leads to novel extraction approaches like ultrasound. SPI extraction has shown more of its functional improvement and is applied widely in practice. In general, comparisons in yield

using sonication to AE (control) were made; the most commonly used method mentioned was ultrasound bath. Moreover, impacts done with UAE in protein properties like functionalities and physiochemical properties have been carefully examined with structural changes, showing a promising landscape in food manufacturing.

- **Extracting proteins from soy slurries and modifications in soy milk**
  Fahmi et al. [9] investigated the effect of ultrasonic treatment (35 kHz, pH 12 for 20, 40, and 60 min at 20 and 40°C) on protein content and rheological behaviours of soymilk extracted from water/soybean slurry before filtration. The result shows a 6.3% rise in protein content compared to the previous in soy milk from extraction. A growth was seen in the yield of UAE based on calculation, as well as in permeability to cell membranes. The rise in permeability is based on its cavitation effect. No significance was seen between the viscosities before and after the treatment (at 2.8 and 3.1% protein concentrations). No change was seen in the consistency coefficient (K) after ultrasound treatment, whereas flow-behaviour indices (n) had a noticeable rise, letting greater portion of water molecules to bind according to Phillips et al. [40]. For further applications in food, both industrial and academic perspectives were requiring more investigations in frequency and power in ultrasound, temperature and time, as well as other possible factors.

- **Protein extraction using UAE from soybean slurry, soy meal and okara samples**
  Considering the needs, further studies using the extraction are conducted. Among those, the pieces of research conducted by Preece et al. [3] and Eze et al. [2] are highlighted in the review.

  The researchers investigated the effect of UAE on an aqueous extraction process from milled soybean slurry and okara samples without pretreatment, with its solubilisation and separation efficiency, for protein, solid and oil [3]. The pH was 7, and the experiment was conducted at 50±1°C using ultrasound (20 kHz, 400 W) for 0.5, 1, 5 and 15 min. Protein yield from soy slurry saw a 10% increase compared to alkaline conditions (pH $\geq$ 8), due to solubility and part of the enhanced separation efficiency, instead of cell disruption. The reduced particle size followed as treatment within 1 min, due to transient cavitation in the liquidus system caused by UAE. For yield in okara, a possible growth was seen in protein, oil and solids after ultrasound treatment; no observable changes were seen in the control. The temperature was not controlled during the treatments since it was negligible. A suboptimal result was obtained compared to alkaline, in conclusion, with improved availability.

  Researchers evaluated the ability of ultrasonication to assist protein yield from defatted okara flour using alkaline phosphate buffer, with the assessment of protein yield, chemical composition, and structural properties of SPI (soy protein isolate) [2]. At pH 12 and 60°C, an extraction yield of 84% w/w was obtained using UAE with alkaline-aqueous solution compared to the alkaline treatment on its own (53.4% w/w). Ultrasound resulted in changes in the secondary structure, which was influenced by treatment time rather than amplitude. Along with the secondary structure change, UAE also increased in the zeta potential and decreased practical sizes, suggesting better emulsifying properties. Similar chemical composition, with high protein (85.5% w/w), and low carbohydrate (3.5% w/w) and low ash (3.8% w/w) profiles were found in both the ultrasound-treated samples and controls.

- **Characteristics and yield of SPI using ultrasonication in comparison to microwave and enzymatic methods**
  To evaluate proteins in soy meal, improvements in SPI by UAE from soy meal in its characteristics, and comparisons in functions with microwave, enzyme and alkaline (AE) extraction were carried out using response surface methodology (RSM) [5]. Maximum yield of SPI under UAE (L/S 15.38:1, amplitude of 51.85%, 21.70°C, pulse 3.49 s, and 11.01 min) found a remarkable improvement over that of rice bran protein using enzyme (14.5%) and microwave (21.1%) methods in contrast. Smaller particle size (27.24±0.33 μm) was observed than in microwave treatment (85.56±0.26 μm).

  Protein purity (91.6±1.08%) Higher content in essential amino acid in SPI using UAE (38.16 g/100 g) was seen than that of microwave (33.33 g/100 g), enzymatically (30.06 g/100 g), and conventionally AE (32.30 g/100 g) in

comparison, with the highest lysine, isoleucine, and histidine content in UAE apart from other methods. Functional behaviours in UAE found a 40–50% increase in solubility, WBC and OBC, EC and FC, and more β-structure in protein secondary structure was found. Additionally, better thermal stability was found. The colour, however, was darker in UAE-treated sample compared to the control. But it was less dark compared to SPIs extracted by microwave and enzyme, which indicates that this may not be a huge problem in appearance to the consumers. Overall, UAE is suggested as a suitable tool for expanding the applications of soy meal in foods.

2.2 Bean proteins from peas and Ganxet, Dolichos, Alfalfa

Interest in beans other than soya with their nutritional and functional values has been shown recently, while some extractions were done for ingredient recovery. Among them, some byproducts, mostly extracted from the seeds of certain plants, were claimed to be underestimated. Following earlier studies on the extraction, most of them made considerable improvements. Novel methods have been suggested.

2.3 Yield of Ganxet with modification on functionality

Ganxet bean, known as (Phaseolus vulgaris L. var. Ganxet), is recognised as a high-quality protein resource with great nutritional value. Easily recognised in its squashed and hooked shape, they are one of the most recognised bean landraces planted in Europe.

Protein from Ganxet beans using UAE (40 kHz, 250 W, 30 or 60 min, mixed with 0.4M NaOH at pH 2, 4, 6, 8, 10) and other different extraction methods were investigated in their yield, solubilized materials (%), protein recovered, with evaluation in its functional properties [19]. Results in UAE from Ganxet beans mixed with Laemmli buffer saw 37.98±0.02, 78.73±4.88% ($p < 0.05$) and 54.58±0.19% in protein yield, protein recovery and the percentage of soluble material, respectively, at pH 5.5 for precipitation. Both pH and time were found to have an association with yield. Lower NaOH concentrations did not see an impact on the amount of GPC (Ganxet protein concentrate) obtained per 100g of raw material, while the protein recovery was significantly better in UAE than in the control. The total protein content of Ganxet beans was calculated as 24.7±0.4%, giving similarity to other legumes like fava beans, compared to 24–29% in previous data [41, 42].

In evaluating the functional behaviours, 0.98±0.10 and 2.33±0.12 of water or oil/g of protein concentrate were seen in WHC and OHC in protein from UAE respectively, at pH 8.0, with the highest EC of 69.4±0.8%. Note that WHC and OHC mentioned in the abstract were different (supposedly a mistake) from the original data therein, where OHC value was replaced by WHC and another value for OHC is assumed. All the data were based on calculations. Both WHC and OHC of the extracted GPC have shown lower values compared to various kidney beans [49]; WHC was similar to that of cowpea protein reported by Ragab et al. [43], while OHC was higher than in hemp seed [50]. This may suggest that it would have better mouth-feel in applications [44], pH 2.0 saw the highest FC (65.0±3.5%), which was comparable to other protein sources like seaweed [45], cowpea [43], or several mung beans [32] therein. At pH 10 FC was higher compared to pH 4-8, while similar FC was found in the compared pH range; time, pH, and the interplay between both impacted FS. Dependence in EC and ES on pH were seen. Moreover, 8.0 was the pH where the highest EC (69.4±0.8%) was achieved, showing similarity to previous results; at pH 2.0, a comparable result in ES of GPC was seen with other plant-based protein emulsions [43], even at its lowest (78.7±1.0%). The foaming ability may let the protein be applied in the whipping process [46]. Therefore, possible applications are explored.

The colour of GPC was measured and compared in detail. L* (refers to lightness), measured as 91.40±1.63 in GPC, was higher than other vegetable-derived proteins such as protein isolates from hempseed meal prepared by both micellization (82.80±0.31) and isoelectric precipitation (56.39±0.29) [50]. The colour of Ganxet protein extracts and their concentrates differed from other vegetable-derived proteins (colour deviation $\Delta E > 3$), such as kidney beans in the Legume family and amaranth [47], while no visible colour deviation ($\Delta E > 3$) was observed in powders derived from common milk proteins in markets [48]. Additionally, no colour measurement has been investigated before in GPC, according to the authors.

2.3.1 Yield and modification of pea protein in functional properties with its biological effect

The pea (Pisum sativum L.), one of the most important crops, is cheaper and more nutritious than most other legumes and food crops, with nourished

protein (233–267 g/kg) and reduced fat (15–20 g/kg) contained. It is also a good source for deriving high-quality protein [26].

The extraction level of pea protein isolate (PPI) from raw pea powder mixed with NaOH using UAE (solid/liquid ratio of 1:11.5 g/ml, ultrasonic amplitude 33.7%, pH 9.6, 25°C, 13.5 min) were carried out, with biological and functional properties of PPI studied at pH 8-10 using same method compared to AE [26]. The extraction level reached 82.6%; changes to the secondary and tertiary protein structure with exposure groups were found; smaller particle size and better dispersion increased solubility, WHC /OHC, FS/EC, and gel formation capacity. Enhancements in biological activity were found in UAE rather than AE. In detail, PPI extracted by UAE showed higher 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity (7.9% higher than that of alkali extraction), doubled hydroxyl radical scavenging capacity, and 55.1% inhibition in increased angiotensin-converting enzyme (ACE) inhibitory activity (8.7% higher). In conclusion, higher efficiency and quality were suggested for certain protein production in the future.

2.3.2 *Yield and modification of Dolichos, functional and antioxidant properties*

Dolichos lablab L., a vine annual plant, whose mature seed is known as 'poor man's protein' due to its enriched protein and affordability, has been widely cultivated in tropical and subtropical areas.

The improvement of UAE (200 W, L/S of 60 ml/g, 30 min, pH 10, 45°C) and the evaluation of functional and antioxidant properties of Dolichos lablab L. protein (DLP) were compared to AE [17]. The protein extracted from its dried bean powder saw a higher extraction rate (69.98%$\pm$1.25%) in UAE than AE (40.95% in extraction rate). Besides, UAE altered the protein secondary structure, which contributes to enhanced functionality and higher antioxidant activity than AE, and the latter is suggested to aid in better health, even alleviate ailments. All of these give evidence on ultrasound-treated DLP in further applications.

The extraction rate decreased by increasing ultrasound power and L/S shown by a negative t-value effect between them; pH, time and temperature had positive synergistic effects. Intensity setting 5.3 W/ml and a temperature of 45°C were chosen to enhance the extraction. Most relationships between UAE conditions and the extraction rate of UDLP (ultrasonicated-DLP) were non-linear, with increasing pH and L/S promoting the yield; no significance was found with time. There was an obvious shrinkage in particle size of UDLP compared to DLP extracted by AE (TDLP); no impacts of UAE were found on the molecular weight distribution of DLP. Moreover, the pI value was found to have no significance between the DLPs using two different extractions. These indicate that the distribution range of particle size in protein dispersion barely changed, even though the particle size of UDLP decreased remarkably.

Both apparent increase found in Tp and ΔH of UDLP after ultrasound assistance indicates better thermal stability obtained after UAE. No remarkable difference was found in the emulsifying activity index (EAI) between the two; the emulsion stability index (ESI) rose significantly in UDLP. The OHC of DLP increased after ultrasound-assisted extraction due to protein conformation. A higher FI (fluorescence index) in UDLP than TDLP and a blue shift in UDLP indicated that Trp (Tryptophan) residues shifted from a hydrophilic environment to a surrounding more hydrophobic or less polar [17]. This probably accounts for the unfolding of protein molecules and the exposure of initially hindered groups supposed to be hydrophobic.

The better surface hydrophobicity of the DLP is suggested, for the amino acid profile, the total amino acid content was obviously higher in UDLP than TDLP. Both their aspartic acid and glutamic acid occupied a high proportion in the amino acid profile, suggesting their acidic characteristics [17]. As a result of changes in secondary structure, the release of certain amino acids during the ultrasonic treatment process is shown, where changes in the content and composition of amino acids may occur.

2.3.3 *Optimisation, comparison, physicochemical, and functional properties of alfalfa protein isolate using UUAAIP technique*

Alfalfa or lucerne (Medicago sativa), cultivated widely around the world as an distinguished forage legume in production, is used in livestock feeding due to its high nutritional quality and adaptability [24]. The great potential for human consumption is claimed due to its high protein content (>250 g kg-1 d.b. (d.b. - dry basis)) found in their leaves. Albumin (main) and some less important proteins (gluten and globulin) are present in alfalfa leaves. The initial sensory attributes and low quality limited its consumption.

Ultrasound-Ultrafiltration-Assisted Alkaline Isoelectric Precipitation, or UUAAIP, a new

technique purposed for the protein isolates extraction, enhancement, improved yield and protein content at the same time, while critical factors in relevance were assessed in the procedure [23]. Maximised both protein yield and content in extraction (solvent/solid material ratio of 43.3 ml/g, flow rate of 9.7 L/h, pH 10.1, 42.5°C, 102 min) from dried alfalfa whole leaves were 14.5 g/100 g and 91.1 g/100 g, respectively. In heat-coagulation extraction (HCE), the highest extraction yield (16.6 g/100 g) appeared with the lowest protein content obtained, which was not comparable with UUAAIP. Optimum protein content (UUAAIP), 91.1 g/100 g, was higher than the value in alkaline isoelectric precipitation extraction (AIPE, 74.5 g/100 g) and HCE(63.9 g/100 g). Functional properties of the protein isolate extracted from UUAAIP extraction under varied temperature (30-50°C) and pH (9-10) have their solubility, WHC and oil-binding capacities improved, but resulted in reduced emulsifying and foaming properties than HCE (pH 7.5 at 85°C) and AIPE (pH 9.0 at 25°C). Improved average molecular weight and colour of the isolate were found.

The interactions of solvent/solid material ratio, pH and time with respect to flow rate clearly showed that the ratio was the most important parameter, which positively impacted the extraction yield from 20 to 45 ml/g. Later, descending yield with respect to the ratio was found due to the driving force gained in the protein in mass transfer, possibly a result of diffusion [35]. Additionally, an increase with time in the extraction yield was seen until 90 min, followed by a drop; at pH 10 to 10.5 was almost equal, though at greater pH a decrease occurred. Protein content, which varied from 60 to 92% depending on different factors, was more significantly affected by solvent/solid material ratio, pH, temperature and time. Time was the most crucial factor, where a growth in protein content was found, probably due to the reason that the mixture crossing through the membrane filtration required a longer time, causing more impurities apart from protein. Average molecular weight (ranged from 71.4 to 84.5 kDa) after UUAAIP treatment was considerably higher than that of AIPE and HCE, matching agreement with the extraction either in alkaline condition [52] or using filtration technique [24].

The highest solubility from UUAAIP (94.7%) at pH 8.0 was the highest compared with the one in AIPE(93.6%) at the same pH and in HCE (92.2%) at pH 9.0. This was similar to the result using alkali in extracting the same protein [51]. Differences among all alfalfa proteins in WHC and OBC were highlighted. Higher WHC of UUAAIP (4.35 g water/g) was found than that of AIPE (3.90 g water/g) and HCE (3.34 g water/g). OBCs were greater than WHCs among all; protein from UUAAIP had the greatest OBC with 4.88 g oil/g, remarkably greater than AIPE (4.27 g oil/g) and HCE (3.95 g oil/g) in their values. Supereme FC and FS of all the extracted alfalfa proteins were found, despite FC of protein from AIPE (521.4%) being the highest, followed by protein from HCE (492.5%) and UUAAIP (365.8%). Similar results were found in the foam stability. However, for the EAI and ES, remarkably lower values were seen in UUAAIP than those in AIPE and HCE, due to the lower saponin content in UUAAIP caused by ultrafiltration on the removal and possibly sonication. But this results in higher L value, lower total phenol and saponin content in UUAAIP protein (68.1; 1.56mg/g and 4.45mg/g) than HCE (59.3; 8.11mg/g and 6.07mg/g) and AIPE (56.7; 7.68mg/g and 8.58mg/g). L value gives better colour; the latter two are known as anti-nutritional factors, with their reduction leading to better taste in sensory. Therefore, UUAAIP is proven as a viable method for alfalfa protein isolates production, which may resolve the restrictions in its consumption.

3 Nuts - pecan protein extraction

Nut protein extraction has drawn concerns in recent years, apart from their oil extraction. Among them pecan is being studied, and with enzymes working together made a profound extraction result.

3.1 Pecan protein

Pecan (Carya illinoinensis (Wangenh.) K. Koch), originated in North America (northern Mexico and the US) and is currently grown in southern parts of China. Enriched in proteins, various amino acids and other bioactive compounds, it draws concerns due to high dietary and healthcare value, which would be promising for application from functional food to pharmaceutical use. The study conducted by Wang et al. [15] aimed to enhance the yield with better properties in pecan protein using synergistic impacts of ultrasound and enzymatic extraction (UE) in protein. Several comparisons in its functional, biological properties were made with ultrasonication (U) and enzymatic method (E) applied separately.

3.1.1 Pecan yield using enzymatic and UAE with functional properties

The highest protein extraction rate (25.51%) was achieved applying UE (intensity 1415.43 W/cm^2, 1%

w/w alkaline proteinase, pH 10.0, 50°C, 15 min) from dried pecan powder mixed with distilled water, compared to U and E. Improvements in emulsifying activity (120.56 m2/g), smaller particle size (326.7 nm), and better dispersion (0.305) were found. Additionally, the result in better solubility (70.77%) was compared with single ultrasound and non-ultrasound methods. The reduction in the size of protein aggregates and the aggregate size of leguminous plant-derived proteins were found in great significance. The aggregation of protein molecules relates to the formation of intermolecular disulfide bridges, which were reduced due to hydroxyl-free radicals. These phenomena could reduce the soluble protein content in the extraction.

3.1.2 Modification of extraction from pecan protein with functional properties under different conditions

The best condition using E (1% w/w alkaline proteinase, pH 10.0, 50°C, 3 h) was found, with only changes in temperature and time (55°C, 15 min) in UE, as the chemical bonds begin to break at 60°C, causing the collapse in molecular structure with their precipitation, leading to a decrease in the solubility and then yield. The dislocation in certain hydrogen and ionic bonds without covalent bond change was seen due to several combined effects (also known in its physical, mechanical and chemical reactions triggered by cavitation) under ultrasonication. Hence, the yield was improved.

Modified UE (400 W, 20 kHz, pH 8-10) changed the secondary and tertiary structure of the pecan protein, with more unfolded structure and exposure of hydrophobic groups and sulfhydryl (SHF) groups in the protein. The change was possibly related to the shift in Trp, similar to the extraction of DLP [17]. Additionally, ultrasound could open the compact structure of protein and lead to conformational changes, as indicated by results in hydrophobicity, SHF groups and particle size distribution. These changes suggest more flexible structures obtained after UAE, exposing a wide range of groups that were buried inside. The change may have a great impact on its functional properties, which possibly be the reason why higher solubility and emulsifying properties were found.

Ultrasonic power (100-400 W) greatly enhanced the extraction yield due to cavitation disrupting cell walls. The power was seen as one of the critical factors in extraction yield, as the mechanical vibration effect of the waves in U provides larger surface area between the matrix and solvent to contact. The result found agreement with Chittapalo et al. [29] and Görgüç et al. [21] in the article, though the plants used varied. The protein extraction yield had escalated from 5-15 min, peaking at 15 min, followed by a dwindle until 25 min. The decrease was seen since the hindered mass transfer effect owing to the enhanced cavitation. Better stability of the protein extracted by UE, for its denaturation temperature was improved from 80.5°C (in E) to 110.1°C. These theoretical bases support UAE as a method for the potential utilisation of pecan protein in industrial practices in food.

4 Cereal Protein

Cereal proteins play a critical part among proteins due to their composition. In this part, extractions and modifications on wheat protein, rice bran protein and Brewer's Spent Grain are studied. Despite their difference, most of the proteins extracted were either difficult to gain or sensitive. Certain ingredient recoveries were considered.

4.1 Wheat Proteins using Ultrasound in Extraction with Size-Exclusion HPLC in Analysis

Experimental parameters affecting sonication (20 kHz, power settings 10–80% (scale 25), ambient temperature and 60°C, 40 and 120 min) determined by size-exclusion (SE) HPLC were investigated, with the extractability, and modulation in the size distribution of total protein in wheat flour demonstrated [28]. Power settings, temperature (30 and 90 sec), operation time and added protease inhibitors were investigated in flour stability.

Ultrasonic energy delivered to the flour sample was the main factor affecting protein extractability and size distribution, either shifting the protein to larger insoluble ones or preventing aggregation during dispersion. The time and power settings both significantly improved protein yield in ultrasonication. On the other hand, an obvious negative effect was found in the interaction between power and time, meaning that the effect of solubility gained due to elongated time (30–90 sec) was no longer very strong at high power settings.

Stability upon re-injection improved due to temperature (deactivation of flour proteases in flour samples at 60°C) and protein inhibitors in the profile obtained from SE-HPLC measurements. The sensitivity of gluten macropolymers was demonstrated in sonication. The proportions of the earliest eluted SE-HPLC fractions were F1 and F2. Below the energy level of UAE required for total protein extraction, a

gentle increase was observed in the F1 area, peaking at 2,400 (sec·%) of ultrasonic energy. Above this energy level, fraction F1, which comprises very large polymers excluded from the column, showed a decrease in favour of F2 in area, then levelled off. Although total protein extraction was not fully achieved, oversonication occurs, as indicated by the decrease in F1. Therefore, limited ultrasonic energy was applied to the sample to maximise the F1 area while achieving total protein extraction. However, the decline in F1 was not seen under the highest power (7,500 sec·%) due to SDS foaming. No overheating was detected in UAE, possibly due to the large solvent volume used.

Enhanced solubility of polymeric protein (sum of F1 and F2 areas) relates to the increase in protein solubility, hence the extractability. Larger polymers originating from SDS-insoluble protein fraction could be unusually sensitive to heat treatment due to the foaming of SDS. The tests in predicting rheological behaviour in SE-HPLC profiles evidenced SDS-soluble / insoluble protein estimation for smaller size distribution. However, contrasting size-distribution ranges may appear in protein polymers (Gpol) from SDS-insoluble fraction. Under the test, relationships between the W index in alveograph and definite amounts from total protein (unextractable or in F1) extract were of great significance and equivalence. The discussion in both SDS-soluble and SDS-insoluble protein extracts is made in terms of the correlation of the index and flour protein content with the fractions of absolute and relative amounts quantified by SE-HPLC from the total. The size distribution of SDS-soluble polymeric protein towards smaller sizes, evidenced by SDS-insoluble protein breakdown in sonication.

4.2 Rice Bran Protein Extraction using Ultrasound and Enzyme

The improvement of UAE from defatted rice bran protein concentrate (DRBPC) using a series of ultrasonic treatments was compared with AE, in terms of yield and functional properties [29]. Under different power levels (40-100 W) at pH 11 for 6-40 min, the yield reached around 0.378 mg/ml within 20 min for most power levels except 40 W. At 40 W, an increase in yield was observed with time. Longer time in conventional alkaline hydrolysis with lower yield (around 0.32 mg/ml) was compared to UAE. The reaction rate constant was associated with intensified ultrasonic power, which shortened the extraction time. The rate of extraction by the conventional and ultrasonic methods decreased exponentially. Thus, the amount of protein changed reasonably. The optimum condition for the preparation of DRBPC using the ultrasonic method (100 W for 5 min) and the conventional method (60 min) was found. No significance was found in DRBPC between ultrasonic (100 W for 5 min) and conventional alkaline treatment in bulk density, foaming and emulsifying stability. In contrast, higher water and oil absorption with lower FC and emulsifying activity, with a lighter brown colour, were found after UAE. In addition, similar profiles of the samples were shown in the Nitrogen Solubility Index (NSI) at pH 4-6 (lowest values).

Later, the extraction of rice bran protein (RBP) from defatted rice bran (DFRB) mixed with water using sonication was compared to AE (pH 10), with protein hydrolysates catalysed by subtilisin A (SPH), Actinase E (APH) and Neutrase 0.8 L (NPH) being investigated [30]. The optimised condition for rice bran protein extraction was claimed to be desirable and practical for relevant plants, with shorter extraction times than conventional methods. Some desired properties in enzyme-treated PH for specific usage were suggested, with the process in production and the degree of hydrolysis control.

A protein yield of 4.73±0.03% was found under ultrasonication (amplitude 76%, 0.99 g/10 ml solid-liquid ratio, at room temperature for 18 min). Hydrolysis using different enzymes was done after the ultrasound treatment. The protein treated by the above three commercialised enzymes is known as protein hydrolysate (PH). The highest degree of hydrolysis was found in SPH (20.03±0.24%), followed by APH (13.84±0.04%) and NPH (5.54±0.07%). Chemical and bio-functional properties were determined. Both scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ACE inhibitory activity saw the highest values in NPH among the PHs, where the latter one was due to the increased hydrophobic amino acid. The highest positive effect in foaming activity (66.25%) was contributed by NPH, followed by APH (57.50%) and SPH (52.50%), with the above suggesting that the rice bran proteins which partially hydrolysed fits better than the non-hydrolysed in food systems.

4.3 Brewer's spent grains protein extraction with functional characteristics

BSG (Brewer's spent grain), a major by-product from brewing industries, is claimed as a cheap and valuable protein source. It is beneficial to intestinal digestion,

alleviating both constipation and diarrhoea due to its glutamine-rich protein, high content of non-cellulosic polysaccharides and smaller portions of β-glucans based on rat studies [31]. Horsin has been seen as the major target protein [33]. Furthermore, high in vitro bioactivities like antioxidant activity and ACE inhibitory activity were seen in BSG peptides, which may expand their application [32].

The idealised protein yield for quantitative extraction from dried BSG (Brewer's spent grain) mixed with 110 mM NaOH was determined with several factors, using tridimensional response surfaces [14]. The yield under the extraction (power 88.2 W/100 ml of extractant, solid-liquid ratio 2.0 g/100 ml, 81.4 min) reached 104.2 mg. Time impacted the most in the extraction, followed by solid-liquid ratio and power, with their negative quadratic effects; all variables in their interactions were unnoteworthy, which could be neglected in evaluation. Higher pH had a considerable improvement in yield, but it was not mentioned in the study. Larger scales are required in further studies.

Impacts of ultrasonic extraction conditions (power, DC and time) on techno-functional properties of protein were evaluated apart from yield improvement for UAE from BSG [6]. Duty cycle (DC), known as a factor in pulsed ultrasound, was described in percentage or as pulsed on/off time. As an enhanced extraction (250 W, DC of 60% (pulsed on/off time 3/2 s), 20 min, 25°C), it reached 86.16% compared to 45.71% in AE without ultrasound. 5.83% higher in β-sheet content while reduced structure in α-helix (by 4.76%), β-turn (by 0.33%) and random coil (by 0.74%) from UAE were compared to the traditional. The changes lead to dissociation and re-aggregation of protein (proved by UV spectra), while also more structural flexibility. The structural flexibility can be reflected in its properties, measured with EAI and ESI, which peaked at 25 min and at 20 min, respectively. The highest value of EAI (40.44$\pm$1.68 m^2/g) and ESI (86.64$\pm$2.38%) was observed under DC of 60% and 40%, EAI and ESI of UAE-BSGP increased by 4.52% and 28.32%, respectively. It indicated that ultrasound treatment had a greater influence on ESI instead of EAI. From 150 to 350 W (power), EAI rose gradually while ESI increased until maximum (81.62$\pm$0.42%) at the power of 250 W, then declined.

The power intensified yield with better extractability until the optimum. Under different DCs, there was a decrease followed by an increasing trend, while no significance rise was found in the enhancement in WAC level from DC 60-100%, indicating that a higher DC contributed little to the WAC level of BSG protein. In addition, protein denaturation may happen under the highest (100%) DC, where the decrease in absorption was found after 80% DC in continuous mode. The decrease suggested that protein aggregation might occur, followed by impacts in protein migration and absorption at the oil-water interface, which lowers EA and ES. This could also be found in a longer operation time, which SHF changes proved. Treatment time of 5 min showed a significantly lower SHF content in BSG, while 10–25 min was unnoteworthy due to their density and correlated structure. A gradual rise of FAC was seen under strengthened power and extended time. However, a decline in FAC after peaking at 3.10$\pm$0.10 g/g appeared under DC of 60% . In addition, improved functionalities in FAC, emulsifying and foaming properties were found in the protein; similar statistics were found between UAE-BSGP and TE-BSGP in WAC. Hydrophobic amino acids were identified by protein absorption intensity (in UV spectra), which is suggested to contribute in WAC/ FAC functionality. Data proved that tyrosine, Trp and phenylalanine contributed to hydrophobicity together. Simultaneously, results from spectroscopy UV–Vis, SHF content and more unfolded protein structure showed that the indeed modified protein structure after UAE.

Hordein (A, B and C) and protein Z have been suggested to be foam-active proteins Therefore, stronger foaming should appear, which was proved by observed maximum FE (112.89$\pm$2.90%) at 250 W and FS (111.26$\pm$0.74%) at 300 W. At 350 W, a slight drop appeared in FS. FE under other conditions saw the drop as well, where it escalated and peaked at 20 min (126.92$\pm$1.41%) before this happened. Moreover, typical inverted V-shape curves in both FE and FS profiles both showed with increasing DCs. The consequence was supposed with protein aggregation. Better SDS-PAGE patterns and more SS suggesting exposure and unfolding of protein structure, which contributed to the enhancement of functional properties of BSG protein. Ultrasound in alliance with NaOH better disrupted the interactions between lignin and protein molecules, which agreed with Tang et al. [14] in data. UAE graphed a promising picture for industrial settings; the modifications evidenced it as an alternative source for future protein supplements.

5 Extraction from defatted sunflower meal

5.1 Extraction yield, physicochemical properties and its function

The research, conducted by Dabbour et al. [1], intended to improve protein yield with lower energy consumption during the extraction from sunflower meal mixed with NaOH at pH 8.0, with details on the better physicochemical and good functional characteristics of the sunflower meal protein isolate (SFPI) after UAE. The highest yield (54.26%) under UAE (220 W/L, 45°C, 15 min) was compared to that of using isoelectric precipitated and ultrafiltration techniques (7.30% and 4.35%), with moderate energy consumption (0.13 kW·h) and protein content of 934.92 g/kg d.b. Lower particle size (627.6 nm) and WHC (0.985g water/g protein), greater bulk density (0.372 g/ml) and OHC (2.06g oil/g protein) were gained at the highest yield as well, compared with the most with Malik et al. [54, 55] in sunflower protein isolate, bulk density in jackfruit seeds [58]. Nevertheless, the bulk density was found to be lower than that of protein isolates (1.4 g/ml) of lentil [56], which may contribute to the moisture content and shape of particles; lower OHC than the protein isolates from safflower (2.77 g oil/g protein) investigated by Ulloa et al. [57] was found.

The highest solubility (74.59), emulsifying activity (EA, 52.45), and ES (50.45%) were found at pH 9.0. Additionally, the highest consumption (0.20 kW·h) was found under 45°C, 25 min; the lowest energy consumption (0.05 kW·h) was achieved at a different condition (80 W/L, 35°C, 5 min). No significance in power density on power consumption was demonstrated. The study suggested a modification of UAE, both simple and economical. Moreover, after being treated by dual-frequency ultrasound, SFPI was recommended for salad dressing and meat products in food formulation applications.

6 Rapeseed

Rapeseed (Brassica napus L.), the third-largest oilseed crop, left its defatted meal as a byproduct rich in protein content (35 - 45%) with well-balanced amino acids and considerably low cost. Moreover, properties of the rapeseed protein in nutrition and functions are represented by two main protein families — cruciferin (12S globulin) and napin (2S albumin) [4]. Less thermal damage was considered while applying to UAE. Since limited information on UAE of rapeseed proteins was given, the importance of its protein release was stressed.

6.1 Proteins in rapeseed with extraction efficiency

The efficiency of rapeseed meal protein (RSP) extraction assisted by ultrasound extraction was generally tested and improved using RSM, with a focus on screening the protein isolate structure for its conformation and properties [4]. Both the yield and nitrogen re-solubility saw an increase (43.3% and 18.13%) with UAE (intensity 0.228 W/cm^2, power 40%, pH 11.71, 41.48 min) compared to AE (control). There was a steady rise in yield with pH and time until reaching its highest level at pH 11.71 and a time of 41.48 min; yield decreases with pH and time after the maximum. Induced cavitation leading to the disruption of plant cells and a smaller particle size was assumed based on the observed trend. A reduction of 6% in hydrophobicity (S0) was noted compared to the control, which could result from the decreased number of exposed nonpolar amino acid residues due to changes in protein structure. Moreover, an increase in SS and a decrease in SHF and fluorescence intensity were found along with changes in FTIR spectra, suggesting protein unfolding and aggregation.

The maximum protein content (4.52 mg/ml) was tested, with the experimental protein value (4.47 mg/ml) being found. Most of the amino acids were comparable to FAO reference criteria [4] and changed markedly after the UAE compared to the control, except histidine, lysine, and Trp. Since lysine and sulphur-containing amino acids together, the result was comparable. However, limiting AA in both extractions was Trp, whose limited solubility and permeability of solvents over time were seen owing to released substances of both insoluble and cytosolic. With good functional properties. Power intensity, power density, and energy dose (Kg/L) increased with increasing output power as energy efficiency reduced; protein yield in sonication was not directly proportional to the input power and energy efficiency. All of the above make it a promising functional protein source for food and pharmaceutical applications.

7 Sesame protein

Sesame (Sesamum indicum L.) seed, regarded as an industrial crops, whose rise has seen in its global production and plantation area, is rich in oil and protein (18–25%) contents [34]. Its bran, a by-product from oil extraction, was undervalued and is concerned recently for its huge protein in recovery. Difficulties may be encountered in conventional extraction due

to its structure, where high fibre compounds tightly bind to the protein. Having regard for the necessity, attempts with a novel extraction method have been made.

7.1 Discussion on yield and properties of sesame

The evaluations of effectiveness using four different novel techniques (including UAE and UAE with enzyme) alongside conventional AE from sesame bran were compared with regard to initial trials on protein and antioxidant compounds recovery [21]. The parameters (enzyme concentrations, pH, ultrasound power, temperature, and time) significantly affected all responses. The UE (836 W, 35 kHz, 43°C, 98 min, pH 9.8, and 1.248 AU/100g enzyme concentration) yielded the highest protein (87.9%), followed by E using alcalase (79.3%), UAE (59.8%), and E using viscozyme L (41.7%). In contrast, some claim that E using viscozyme L yields more protein than alcalase from sesame cake (Latif & Anwar, 2011) and flaxseed. Protein recovery, total phenolic compounds (TPCs), and antioxidant capacities were enhanced by UAE. Optimisation, treatment comparison, different techniques, and antioxidant properties were taken into consideration. The highest TPC (6.61mg GAE/g) was found in E using alcalase. With ultrasound, TPC was lower in extraction due to the adverse effects of its waves on polyphenols at high ultrasound powers. TPC and AC (antioxidant capacity) of DPPH and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) initially increased with operation time until reaching maximum levels for each response and then began to decrease.

As the gains of enzyme concentration and process temperature, the protein yield also rose and almost levelled off at 1.8 AU/100 g and 40°C, respectively. However, protein yield increased with the ultrasound power intensifying at once, indicating its potentially high phenolic compound and antioxidant capacity values. Huge gaps and holes on the external surface of the UE-treated bran suggested the growing number of cavitation bubbles [38]. In addition, following protein yield and antioxidant results, alcalase destroyed the structure more than viscozyme L.

Apart from the maximised condition, independent factors (enzyme concentrations, pH, ultrasound power, temperature and time) showed their significance on all the responses from 18 groups. However, a declining trend with longer extraction time suggested that extended treatment may cause the degradation of bioactive components. The result in SDS analysis from optimised UE indicates the presence of smaller protein subunits and possibly bioactive peptides. Apart from alcalase-assisted extracts with bands smaller than 9 kDa, similar profiles containing 7 major bands (9-70 kDa) were observed in the analysis of both their native and denatured form. This was almost the same as in rice bran PHs after pepsin-trypsin digestion. During the process, macroproteins digested into polypeptides and into smaller peptide fragments in further steps [30]. Additionally, alcalase in the study [59] had the highest hydrolysis capability among the preparations of enzymes. Therefore, treated by alcalase, the high-value PH can be used as value-added ingredients in various food formulations.

8 Protein from perilla seed

Perilla seed meal, originating from perilla seed oil extraction, is an important agricultural byproduct for its enriched content of bioactive molecules such as proteins, fats and phenolic compounds [27]. The seeds are seen as a food source among the tribal communities in China, cutting across age and economic status; development and utilisation of these bioactive molecules received considerable attention around 2012 to raise the profitability [27]. However, little study has been done at the time.

8.1 Effects of parameters on the yield of protein extraction

Improved UAE with alkaline for proteins in dried perilla seed meal and assessment of the protein quality were conducted [27]. Extraction using UAE (61 W, 40°C, 12 min, L/S 40 ml/g) from defatted perilla meal yielded the highest (10.77%) protein content. The yield of extracted proteins first rose and then decreased as the L/S rose from 20 to 40 ml/g. A linear decline in yield with temperature from 40 to 60°C indicates that a higher yield may be obtained below 40°C. The interaction between time and L/S shows a remarkable effect on protein yield, with time having a more significant effect than L/S. Note that although phytics acids and phenolic compounds were mentioned in the seed, the compounds were removed before protein extraction. Other parameters such as pH, ionic strength, salt or solvent type, extraction time and the presence of cross-linking components affect the solubility of proteins. Extraction, isolation and fractionation procedures may differ depending on the use. Alkaline aqueous extraction followed by isoelectric precipitation with pH 4.0-5.0 is applied with fractionation performed for food applications.

9 Conclusion

A variety of efficiency in extractions with better modifications have been carried out, evidencing UAE as a method for protein preparation and product enhancement in industrialised foods. Ingredients recovery in extraction and novel protein sources are widely considered to reduce costs and waste. Enhanced efficiency with a few energy concerns; structural changes due to both in preparations and during treatments have been mentioned. The functional behaviours measured in WHC, OHC, ES, EC, FS, FC and hydrophobicity have been discussed. UAE under different conditions have been investigated, with suboptimal results reached. Enzymes in alignment with UAE for some cereals, seeds and nuts [15, 28], while UUAAIP in yield and function improvement around alfalfa leaf protein [23], were evaluated. It is noticeable that few of them have applied the pulsed ultrasound, which appeared in more recent articles in conventional applied ultrasound [5, 6]. In addition, extractions from complex structure or fibril structure (Figure 3)seen in matrices or slurries were alleviated by UAE. Cell disruption in UAE may not be favourable during exact treatments, especially for extractions of sensitive proteins. The adverse impacts in UAE have been proved as a consequence of the cavitation effect mostly, with diffusion mentioned.

A definite number of yields were found to be related to operation time instead of power in UAE. In some, however, power matters the most, or time can have adverse impacts. Combined methods could lead to inspiration for reducing energy consumption for greener extraction purposes with better protein extractability, indicating a trend in future studies.

Data Availability Statement

Data will be made available on request.

Funding

This work was supported without any funding.

Conflicts of Interest

The authors declare no conflicts of interest.

Ethical Approval and Consent to Participate

Not applicable.

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