

## Protein Extraction from Plant-Based Waste Using Green Extraction Technology

Mishra N.<sup>1\*</sup> and Jyoti Y.K.<sup>2</sup>

<sup>1</sup>Dr Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar

<sup>2</sup>Department of Agricultural Processing and Food Engineering, OUAT, Bhubaneswar, Odisha

\*Corresponding author

Email address: [nikitamishra1505@gmail.com](mailto:nikitamishra1505@gmail.com)

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### Abstract

The worldwide food production industry produces vast amounts of plant waste material each year ranging from fruit peels to vegetable pomace, and from oilseed press cake to cereal bran. Embedded within all this wasted biomass is an under-utilized source of valuable proteins. Although there exist well-established procedures for their separation from plant materials, the current methods employ numerous amounts of harmful organic solvents, huge energy requirements, and severe conditions that damage both proteins and the environment. A paradigm shift to greener extraction techniques is required in order to exploit biomass as an excellent source of valuable proteins.

**Keywords:** Plant waste biomass, Protein recovery, Green extraction techniques, Agro-industrial by-products, Sustainable food processing

### 1. Introduction: The Hidden Value in Plant Waste

The annual amount of plant biomass wasted in the agri-food sector worldwide amounts to hundreds of millions of tones. Just a few examples of such industrial-scale by-products include apple pomace, which comes from juicing; soybean meal, produced during oil pressing; tomato seeds and peel, from producing sauces; brewers' spent grain; and sugar beet pulp. All of these biomass streams contribute not only to the problem of greenhouse gas emissions, landfilling, and water pollution but also constitute a huge economic loss.

Among others, proteins are some of the most precious bioactive molecules contained in the waste streams mentioned above. The increasing demand for plant proteins derives from the rising global population, flexitarianism, and veganism, as well as the need to seek alternative ways to produce proteins in order to protect the environment. The protein

composition in agro-industrial waste can vary between 5%, as seen in fruit pomaces, and over 40%, as seen in oilseed press cakes.

Extracting proteins from such waste streams is quite a challenging task. The proteins are embedded in complicated cellular matrices, and at times may be associated with polysaccharides, phenolic components, and lipids. The extraction procedures conventionally practiced include alkaline solubility and precipitation using their isoelectric point, and/or solvent-based techniques which work but entail considerable limitations, such as the employment of toxic solvents, the formation of acidic/alkaline effluents, and the denaturation of proteins due to heating processes. This is where green extraction technology steps into the scene.

## 2. Green Extraction

The concept of "green extraction" was formally articulated in the context of natural product chemistry, drawing from the twelve principles of green chemistry. In the context of protein extraction, green extraction refers to processes that:

- Minimize or eliminate hazardous solvents, replacing them with water, ethanol, or other GRAS (Generally Recognized as Safe) solvents
- Reduce energy consumption through optimized process conditions
- Maximize extraction yield and protein quality simultaneously
- Generate minimal waste or convert by-products into useful products
- Preserve biological functionality of the extracted proteins

The green extraction paradigm is not a single technology but a philosophy encompassing several innovative approaches, each suited to different waste streams and target applications.

## 3. Green Extraction Technologies

### 3.1 Ultrasound-Assisted Extraction (UAE)

The use of ultrasound in extracting proteins is based on the application of high-frequency sound waves (20–100 kHz), which cause acoustic cavitation within the liquid medium. The explosion of microbubbles leads to the creation of very high local pressures and temperatures, resulting in the disintegration of the cell wall structure and subsequent release of intracellular proteins into the solvent.

Some of the key benefits of using UAE to extract proteins from biomass include shorter times of extraction (minutes rather than hours), ability to perform extractions at relatively low temperatures, thus reducing denaturation. Various studies have shown an increased protein

yield of between 15 and 40 percent when compared to traditional stirring techniques. The functionality of proteins extracted with UAE was also shown not to be compromised since parameters such as solubility, emulsification, and foaming were improved. The main drawback of UAE is associated with the scalability of the technology, which is challenging due to the difficulties in upscaling laboratory ultrasonic probes. Nonetheless, ultrasonic reactors have been developed.

### 3.2 Enzyme-Assisted Extraction (EAE)

The enzyme-assisted extraction takes advantage of the highly specific and effective action of biological enzymes for hydrolysis of cell wall compounds consisting mainly of cellulose, hemicellulose, and pectin which entrap proteins in plant materials. Among common enzymes used in EAE techniques are cellulases, hemicelluloses, proteases, pectinases, and xylanases, each or in combinations of different types.

EAE works on selective degradation of non-proteinaceous cellular matrix to yield proteins without necessitating extreme pH change or solvent use, hence making EAE especially suitable for extraction of protein from high-fiber materials like brewer's spent grains, sugar beet pulp, and hemp press cakes. Moreover, proteins extracted using this technique are found to be more soluble and digestible.

A notable advantage of EAE is its compatibility with mild, aqueous conditions at near-neutral pH, which preserves native protein structure and bioactivity. The main limitations are the cost of commercial enzyme preparations and the need to inactivate enzymes post-extraction to prevent over-hydrolysis. Research into cheap, microbially produced enzyme cocktails tailored to specific waste streams is actively addressing the cost barrier.

### 3.3 Pulsed Electric Field (PEF) Technology

Pulsed electric field technology involves the exposure of the plant material to short pulses of high electric energy (from 1 to 50 kV/cm for a period from micro- to milliseconds). Electric field creates electroporation – temporary or even irreversible openings in cell membranes which enables leakage of intracellular proteins and other biological macromolecules into the extracting medium.

PEF is a non-thermal process, which makes it particularly interesting for heat-labile proteins. This technology has been successfully employed in the extraction of fruit juices from potatoes (the waste product from starch production), microalgae, and several vegetable waste products. Energy consumption is relatively low when compared to thermal processes and can also be used for creating continuous processing lines. The PEF pre-treatment is usually

complemented by other "green" processes, for example PEF treatment followed by centrifugation or ultrafiltration.

### **3.4 Supercritical Fluid Extraction (SFE)**

Supercritical fluid extraction (SFE) is mainly associated with the extraction of lipids (defatting) and not of proteins. However, SFE makes a huge contribution towards greening the protein extraction process. By extracting lipids from oilseed cakes using supercritical CO<sub>2</sub> before conducting any protein extraction, the process ensures that protein fractions produced are not affected by the use of any harmful chemical substances such as hexane, which is the industry-standard solvent for removing lipids from oilseed cakes.

Defatting meals using hexane-free methods through SFE is one of the key steps towards greening the process. Proteins extracted from hexane-defatted meals exhibit reduced solubility and functionality because their structures may be altered by heat and exposure to the solvent. Proteins isolated using SFE always possess higher functionality levels than those extracted using hexane. The only limitation of SFE is the relatively high initial costs of the equipment used.

### **3.5 Aqueous Two-Phase Systems (ATPS)**

Aqueous two-phase systems refer to the spontaneous generation of two immiscible aqueous phases where a particular combination of polymers (such as polyethylene glycol and dextran) or polymers with salts (such as PEG and potassium phosphate) is dissolved above the critical concentrations. The surface properties of the proteins enable their separation into two phases.

Aqueous two-phase systems offer an environmentally friendly approach to protein isolation and separation through a completely aqueous process that eliminates the use of any organic solvent and extreme pH values. ATPS has been used effectively in the isolation and partial purification of proteins from the water associated with potato processing, green leaf material, and legumes. The incorporation of bio-derived compounds like starch and ionic liquids instead of petroleum-based polymers makes the technique even more sustainable.

### **3.6 Fermentation-Assisted Extraction**

The use of solid-state fermentation or submerged fermentation employing generally safe microorganisms such as lactobacillus species, *Aspergillus oryzae*, and *Rhizopus oligosporus* provides a biological means of protein release. Enzymes produced by the microorganisms in the course of fermentation hydrolyze cell wall polysaccharides, decrease anti-nutritional compounds such as phytate and tannin contents, and sometimes elevate the total protein level through microbial biomass growth.

Fermentation becomes especially beneficial for the conversion of lignocellulosic materials whose proteins are difficult to isolate because of the presence of fibers that do not allow easy extraction. Various fermented cereal brans, leguminous hulls, and oil seed cakes have been found to exhibit higher protein solubility after microbial fermentation. The method requires low energy investment, employs inexpensive microbial inocula, and may result in simultaneous production of prebiotics and organic acids.

## **4. Plant-Waste Materials and Protein Extraction**

### **4.1 Press Cakes from Oilseed Meals**

Press cakes obtained after extraction from sunflower seeds, rape seeds, hemp seeds, flax seeds, or sesame seeds contain up to 20-40% proteins. At present, press cakes serve primarily as animal feed products of low value. Green extraction makes it possible to turn this material into valuable food protein concentrate or isolate.

### **4.2 Legume Processing Residues**

The production of soy milk, tofu, and other legume-based foods generates okara (soy pulp) as a byproduct. Okara contains 20–30% protein on a dry-weight basis and is produced in millions of tons annually in Asia. EAE and fermentation approaches have shown particular promise in valorizing okara proteins due to its high moisture content and fiber matrix.

### **4.3 Cereal Bran and Brewers' Spent Grain**

Wheat bran, rice bran, and brewers spent grain the fibrous residue from beer brewing collectively represent enormous volumes of protein-rich co-products. Brewers' spent grain contains approximately 20–25% protein and is produced at a rate of roughly 30 million tons per year globally. Ultrasound and enzyme-assisted processes have demonstrated effective protein extraction from these matrices.

### **4.4 Fruit and Vegetable Processing Residues**

Tomato pomace, apple pomace, grape marc, and potato pulp contain more modest protein levels (5–15%) but are generated in such large volumes that even modest protein recovery represents a significant resource. The challenge here is the high moisture content and rapid perishability of fresh residues, which necessitates rapid processing or drying prior to 4.3 Wheat Bran, Rice Bran, and Brewers' Spent Grains

Wheat bran, rice bran, and brewers' spent grains, which are the byproducts of beer production, constitute massive quantities of protein-filled byproducts. The protein content of brewers' spent grain is around 20–25%, and it is made available on an annual global basis in a

quantity of about 30 million tons. Protein extraction from these sources using ultrasound and enzymes has been successful.

#### **4.4 Plant and Vegetable Residues**

Tomato pomace, apple pomace, grape marc, and potato pulp have lower amounts of protein (5-15%), although they are produced in very large quantities, hence their potential as protein sources. However, the main difficulty with this source is the high level of moisture in the fresh material and its high perishability, requiring extraction shortly after collection or drying extraction.

### **5. Challenges and Future Directions**

Nevertheless, despite significant advances in the field, a number of issues should be solved to enable green extraction to become commercially competitive and gain traction in large-scale production. One of the crucial problems is the scaling-up of existing extraction techniques from lab to pilot scale. For instance, scaling-up of ultrasound-assisted, PEF-assisted, and enzyme-assisted extractions all require extensive engineering effort. Continuous flow processes seem to be more promising than batch systems. Many green extraction techniques lack economic competitiveness against traditional extraction procedures due to higher prices for enzymes, equipment, and longer time of operation. It is critical to carry out detailed techno-economic and life-cycle assessment to select the best possible approach. Regulatory issues should be considered carefully. Innovative protein extraction procedures may result in production of proteins with modified structure and accompanied by new substances used during the procedure; thus, regulatory approval is required prior to using these novel products in foods.

Protein functionality and allergenicity should be examined thoroughly. Modification of proteins in green extraction processes can affect their functionality in various manners, and the role of accompanying substances, such as polyphenols, requires investigation. In terms of future development perspectives, the application of several green technologies in the cascade biorefineries approach is most likely to yield good results. Single waste streams from plants can undergo multiple processes to isolate proteins, polyphenols, dietary fiber, and lipids, all of which may have potential applications in food/feed/cosmetic/pharmaceutical industries. The involvement of digital technology and intensification techniques, along with circular economy principles, will be crucial for achieving this objective.

### **6. Conclusion**

The use of green technologies to recover proteins from plant-based waste presents an opportunity that brings together issues of sustainable development, economic efficiency, and

innovation in the food industry. Such approaches as ultrasound-assisted extraction, enzyme-assisted extraction, pulsed electric fields, supercritical fluid treatment, aqueous two-phase systems, and fermentation have unique advantages and should be used selectively according to specific conditions. What is more important is the scale of benefits that can be gained by implementing these technologies: the transformation of agricultural and food processing waste into quality protein ingredients would decrease the amount of wastage and environmental impact of the industry significantly, promote further progress in bioeconomy, and provide new income opportunities.

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