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Review

Downstream Processing, Functional Properties, and Applications of Extracted Proteins from Nonconventional Sources for Improving Food Sustainability

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ABSTRACT: The rising demand for sustainable protein alternatives has encouraged processors to increase their exploration of nonconventional protein sources. Environmental concerns, health issues, and projected population growth to 8.5 billion by 2030 also trigger their utilization. Traditional protein sources like meat and dairy face limitations in meeting these challenges. This review extensively explores the various nonconventional protein sources, such as microalgae, insects, plant leaves, microbial proteins, mushrooms, and jellyfish. These nonconventional protein sources have advantages such as low cost, environmentally friendly production methods, and nutritional benefits. The downstream processing methods, like ultrasound-assisted extraction and high-pressure techniques, help improve protein recovery and alter their functional properties. Furthermore, integrating nonconventional proteins into food systems offers an opportunity to tackle sustainability challenges linked to traditional animal farming. This enables the food industry to decrease environmental impact and bolster global food security.

KEYWORDS: *nonconventional proteins, downstream protein extraction, insects, leaf proteins, mushroom proteins*

INTRODUCTION

In recent years, there has been significant growth in the demand for protein, with around 1 billion people currently experiencing insufficient protein intake.[1] The increase in worldwide protein consumption is not only a consequence of a growing population but is also impacted by economic factors like increased income levels, urbanization, and aging populations. Additionally, traditional protein sources, i.e., animal proteins, are expected to be insufficient to meet the increasing demand for protein in the future.[2] The importance of dietary proteins in promoting healthy aging is becoming more acknowledged, and individual wealth is increasing in many developing countries, leading to a rise in the worldwide demand for protein. Various nonconventional protein sources are available, ranging from plant-based options such as grains to traditional sources like meat and dairy products.[1] Protein sources and their various types vary significantly across geographical locations. Figure 1 represents the various traditional sources of protein consumed worldwide.[3]

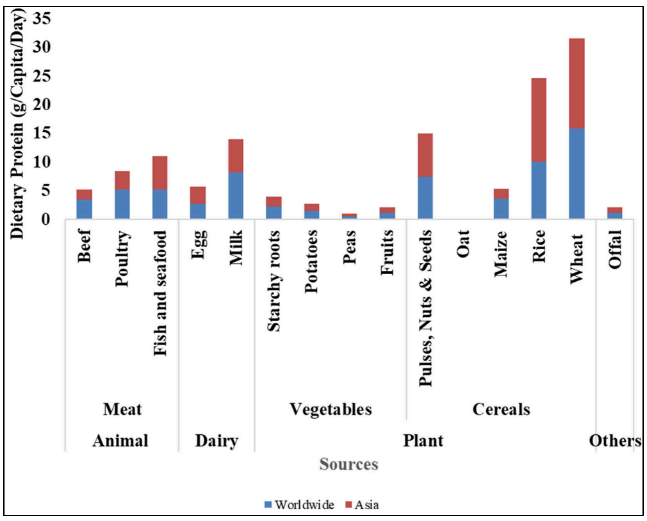

Figure 1. Graphical representation of traditional protein intake sources and future perspective.

Nonmainstream dietary protein sources, including insects, microalgae, and single-cell proteins (SCPs), are increasingly being researched due to their potential to address global food security challenges and provide sustainable alternatives to traditional animal-based proteins. This is because they have a reduced environmental impact while being highly nutritious, hence meeting protein demands worldwide. Insects like crickets and mealworms have a high protein content and a smaller ecological footprint than traditional livestock.[4] Algae, such as spirulina, contain essential amino acids and can be easily cultured under various conditions, making it a sustainable source of protein.[5] Single-cell proteins obtained from microbial biomass provide an efficient solution by converting low-value substrates into valuable high-quality

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protein.[6] To enhance the global supply of protein sources, reduce reliance on traditional animal-based meat, and minimize environmental impacts, including nonconventional protein sources is necessary.

Plant-based proteins, particularly those obtained from microalgae, are growing more popular in the food and feed industries as sustainable alternatives to animal-derived proteins. Microalgae have been identified for their efficient photosynthesis, efficient carbon dioxide (CO_2) and ammonium (NH_4^+) conversion into protein, fast growth and development, and smaller land-use footprint than more traditional protein sources.[7,8] Due to protein insufficiency, spirulina (*Arthrospira platensis*), chlorella (*Chlorella pyrenoidosa*), and green algae (*Dunaliella*) have been widely consumed in various regions.[1] Red and green seaweeds are widely recognized for their high protein content and amino acid profiles, comparable to egg and soybean proteins.[9] Plant leaves are an ideal substitute for proteins because they are inexpensive and widely available, especially in countries that have limited choices for animal-based protein sources. Plant leaves are a more cost-effective source of protein compared to other components with high protein concentrations.[10,11]

Insects are also considered a rich source of high-quality protein and contain all the essential amino acids.[12] The nutritional value of insect proteins varies depending on species, geographical location, and growth stage.[13] The use of microbial protein from bacteria and fungi, including yeasts and filamentous fungi, as food has been in practice since World War I. However, the widespread adoption of microbial protein as a food ingredient has been limited by high costs, subpar product quality, and challenges in their extraction.[14] The jellyfish (*Acromitus hardenbergi*) is an attractive potential source of dietary protein owing to its nutritional profile.[15] Mediterranean jellyfish are considered a potential new food source, and research has demonstrated that thermal processing techniques can alter their protein and phenolic contents.[16]

The processing industry aims to transition to environmentally friendly "green" protein extraction methods due to the associated high environment and consumer safety concerns with conventional processing techniques.[17−20] Green extraction technologies, such as ultrasonic-assisted extraction (UAE) and high-pressure extraction (HPE), have been developed as advanced methods for protein extraction due to their high efficacy and sustainability. UAE employs high-frequency sound waves generated by piezoelectric or magnetostrictive transducers and sonication power. The duration of its application considerably impacts protein extraction efficiency. This method accelerates extraction by generating substantial shear stresses via acoustic cavitation, leading to cell disruption.[21−24] HPE enhances mass transfer and extraction efficiency by breaking biological structures, which reduces extraction times and solvent consumption.[25] Extensive examinations have been made to determine the efficacy of high-pressure technology in extracting plant proteins for application in the food and medicine sector.[26] Nonthermal extraction techniques such as pulsed electric field and high voltage electrical discharge provide alternate methods for extracting cellular components. Cold atmospheric pressure plasma improves the extraction process by employing oxidative stress and electroporation mechanisms as a pretreatment method.[27−30]

This review aims to present nonconventional protein sources such as microalgae, insects, plant leaves, microbial products, fungi, and mushrooms in detail and explore their potential to be used in food products to govern the growing global need for a diet high in protein. This article also focuses on the increasing use of environmentally friendly protein extraction technologies from nonconventional or alternative sources. The aim is to improve extraction efficiency, reduce costs, and achieve sustainability goals for protein extraction. Techno-functional properties of proteins and their way of promoting nonconventional protein sources for making meat substitutes and cereal-based food items like bread, pasta, cookies, and snacks have also been extensively explored.

NONCONVENTIONAL PROTEIN SOURCES

Microalgae. The food and feed industries are investigating plant-based proteins since they are more sustainable than traditionally used animal-based sources. Microalgae are an excellent choice as they can potentially deliver considerable protein in a relatively small land area. Research reported that microalgae could account for 18% of the protein supply by the middle of the century, resulting in a more diversified protein market.[31] Microalgae offer a high-quality amino acid profile suitable for human nutrition, with protein levels ranging from 20% to 70% depending on species and growing conditions.[32] The potential application of proteins from microalgae presents important opportunities for the food sector. These unicellular organisms can grow in a variety of conditions and can supply a community's protein demands, helping to ensure food security.[33] Autotrophic microalgae use ambient resources while converting basic chemicals such as carbon dioxide (CO_2) and ammonium (NH_3) into protein. They have a fast growth rate and efficient photosynthesis, which results in the substantial production of glucose, protein, and fat in a relatively short period.[7,8] Seaweed, or macroalgae, is a sustainable source of underutilized protein, with red and green seaweeds being high in protein and classified as sea vegetables. Their protein compositions vary by region and harvest season, with amino acid profiles often compared to eggs and soybeans.[9] Numerous types of seaweed, including nori and wakame, are currently consumed as food. However, there is also potential to use various seaweed varieties as a source material for protein extraction and isolation to produce pure protein for use in food and feed.

Europe is a major producer of spirulina and chlorella, recognized for their high levels of n-3 long-chain fatty acids.[34,5] These microalgae show promise in substituting for some soybean meal in animal feed, providing up to 630 g of protein per/kg of dry matter and a composition of indole-3-acetic acid similar to animal-derived protein.[35] Spirulina (*arthrospira*) contains all nine essential amino acids, making them complete proteins ideal for vegan diets.[36] Chlorella, a well-recognized type of microalgae, contains high levels of carotenoids, essential amino acids, and significant protein content in its powdered form.[37]

During processing, chlorella is commonly mixed with algae (*Nostoc sphaeroides*), which improves its nutritional profile.[38] The amino acid content of chlorella and spirulina matches the defined requirements by international organizations such as WHO/FAO/UN.[39] Microalgae cultivars grow much faster than traditional plant protein crops, producing 15−30 tonnes of dry biomass per unit area, exceeding soybean and wheat crops.[40] Hence, microalgae, with their diverse species and beneficial properties, have the potential to transform the protein industry by providing sustainable alternatives to traditional protein sources while also lowering environmental

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impacts associated with conventional agriculture and livestock farming.[31]

However, microalgae protein extraction requires energy-intensive downstream processing. Energy requirements for harvesting and processing microalgae biomass could be high, particularly under conventional conditions. For instance, the energy required for algal cultivation under autotrophic conditions is estimated to be ∼18.7 MJ/kg-dw, whereas mixotrophic conditions reduce this by about 11.5 MJ/kg-dw, implying potential for more sustainable practices.[41] Scaling up microalgae-based protein production, low biomass productivity under autotrophic conditions, low extractability due to tough cell walls, undesirable flavors and aromas in products, and intense downstream operations characterized by high levels of energy use.[42] The most commercial production comes from phototrophic open raceway ponds with an average yield of 2.5 kg-dw m^{-2} y^{-1}. However, closed photobioreactor systems may attain up to 8.5 g-dw m^{-2} y^{-1}.[43]

Further improvements are possible if mixotrophic or heterotrophic growth modes are employed, leading to higher productivity due to increased biomass compared to autotrophic systems while consuming less energy; mixotrophic cultivation could potentially achieve up to 17 kg-dw m^{-2} y^{-1} with reduced energy demand relative to its autotropic counterpart.[41] *Dunaliella salina* and *Tetraselmis suecia* have intricate extracellular matrices, making them suitable for large-scale protein extraction despite their lower protein contents.[43,44] Biomass inclusion in foods is also limited to 2−10% due to chlorophyll and fishy odors.[42] In comparison, these drawbacks can be reduced by mixotrophic cultivation.[42] Wet biomass could help alleviate high downstream processing costs due to low cell densities.[42]

Insect Proteins. Insect intake has been deeply rooted in the human diet throughout history, as shown by the consumption of insect eggs, larvae, pupae, and adults since ancient times.[45] Cultural differences among societies affect insect consumption as an alternative protein source. Eastern, African, and Latin American cultures have a long history of eating about 2,000 edible insect species, strongly integrated into their culinary traditions.[46] The nutritional value of edible insects varies greatly based on their geographic origin, developmental stage, and food intake habits. Significant changes in nutritional characteristics can also be identified between different insect species within the same genus.[12] Comparative analyses showed that insects have a higher total protein content and a broader spectrum of essential amino acids, making them nutritionally superior. Insect proteins have a significant protein content, with essential amino acids ranging from 50% to 80%.[13] Many insect varieties support the claim that insects constitute 95% of the animal kingdom's biomass.[47]

Edible insect protein meets WHO amino acid standards and has shown better human digestibility (76−98%) than plant proteins and slightly less than meat and egg white (100%).[48] Research has shown that insect flour from edible insects like *Tenebrio molitor*, *Allomyrina dichotoma*, and *Protaetia brevitarsis* contains 49% − 85% protein with essential amino acids and good emulsification properties.[49,50] *Gryllidae* species have 63% protein-containing essential amino acids such as phenylalanine, tyrosine, tryptophan, lysine, and threonine. The sustainability of proteins in insects is supported by their excellent feed conversion efficiency, high reproductive rates, and short life cycles.[51] They offer a sustainable alternative to protein supply because of their exceptional feed conversion efficiency, high reproduction rates, and short life periods.[12]

In addition to their nutritional value, insects give environmental advantages by emitting less greenhouse gases and ammonia than traditional farm animals.[52,53] Insects can survive on various food sources, including processed food waste, reducing the environmental impact of organic waste disposal. The technological feasibility and functional attributes of insect-derived proteins in food applications are still under investigation.[54] Crickets, locusts, and grasshoppers (orthoptera) have the most protein (61% dry basis), followed by damselflies and dragonflies (55% protein) and termites (35% protein),[55] as shown in Figure 2.

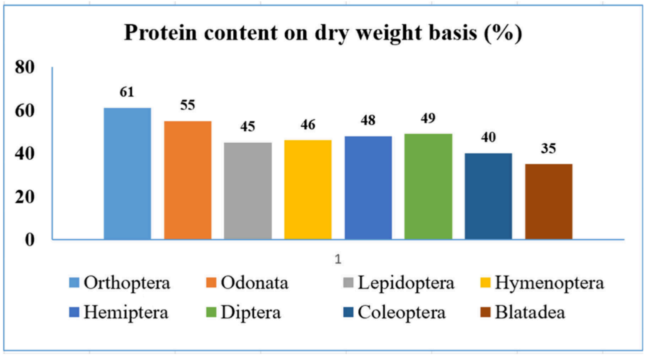

Figure 2. Pictorial representation of protein contents of the eight widely used edible insects.

One of the major challenges to extracting insect proteins is the complexity of the extraction process. Unlike plant proteins, edible insects have high lipid content (13%−53% w/w), necessitating solvent-pretreatment methods such as hexane or ethanol. This can lead to loss of prolamins and concerns about residual solvents in protein fractions. Freezing, followed by milling and air classification, are techniques used to enhance protein yields and produce solvent-free extracts.[56] The other main challenge is the exoskeleton composed of hard chitin, which affects protein extractability and digestibility.[57] Commonly, extraction involves pretreatment, defatting, solubilization of proteins, purification, and drying, with typical alkaline extraction and isoelectric precipitation processes. Ultrasound-assisted extraction shows promise for improving yield, while enzymatic hydrolysis using mammalian or microbial enzymes needs further evaluation, which has not yet been thoroughly studied.[47,48] Another challenge regards economic feasibility given the costs involved in rearing insects, including space requirements, feed input, labor cost, etc.[58] There is also a significant hurdle associated with consumer acceptance since many customers from developed countries already dislike eating insects as foods.[59] Therefore, successfully incorporating these sources into mainstream food products requires overcoming these impediments.

Plant Leaf Proteins. Traditionally, plant-based proteins have been obtained from seeds and other plant parts, such as bran and seed coat.[10] Leaves, as an alternative plant-based protein, are considered a highly affordable and abundant source of nutrition for both humans and animals, particularly in underdeveloped nations.[11] Approximately 80% of the proteins in plant leaves are located in the chloroplasts.[60] Furthermore, food safety and functional characteristics linked to leaf proteins have also been elucidated for some plants.[61]

Previous research has shown that a protein concentrate from jackfruit plant leaves has antioxidant properties and useful functionality.[62] Water hyacinth is one of the most studied plants because its leaves contain up to 50% protein and 17 of the 21 essential amino acids.[63] In 2016, a study evaluated six plant leaf proteins as prospective protein supplements, of which pumpkin leaves exhibited the highest protein concentration, 11.75%, followed by amaranth, 10.7%.[64] In recent studies, mulberry leaf protein has improved gut bacteria balances and reduced inflammation, which are important in treating ulcerative colitis.[65] *Moringa oleifera* leaves have been found as a potential protein source, with protein concentrations ranging from 23.0% to 43.5% in dry matter, which can substitute for traditional protein sources.[66]

Using green leaves as a human protein source presents several challenges, including low protein content and bioavailability, impurities affecting color, flavor, and mouthfeel, as well as the presence of antinutrients and toxic substances.[67] Nonprotein compounds such as fibers, polyphenols, organic acids, minerals, and lipids often coexist within leaf tissues, inhibiting protein extraction and functionality.[68] These nutrients may negatively affect nutrient absorption rates,[69] while tannins, oxalates, and cyanide may harm health.[70] The boiling process can reduce them by 24−76%, while other processing techniques like frying, boiling, drying, or fermenting can improve the quality of proteins.[70] In addition, dietary fibers, among other nonprotein compounds, could complicate matters of isolation, thus forcing harsher extraction conditions that might ultimately affect the quality of proteins in question.[71] Therefore, it is important to ensure that the method used in extraction meets safety requirements and gives residues-free product.[72] High temperatures or alkaline conditions can adversely affect protein attributes during extraction,[73] meaning optimal extraction should guarantee high yield, good quality, scale-up potential, and economic viability. The level of leaf maturity affects protein extraction as reduced amino acid levels are found in senescent leaves, along with varying quantities of antinutrients.[74] Therefore, moisture loss within the leaves and the associated potential for spoilage require proper drying or rapid processing to maintain protein quality.[75,10]

Microbial Proteins. Microbial proteins are a well-established source of proteins, primarily from bacteria and fungi, including yeasts and molds. However, the widespread use of microbial proteins in human and animal feed is hindered by high production costs, poor product quality, high nucleic acid content, and inefficient protein extraction techniques.[14] A study explored autotrophic hydrogen-oxidizing bacteria because of their ability to use nitrogen from various human activities, such as agriculture and livestock practices.[76] Microbes can be a sustainable protein source for human food since they can synthesize large amounts of protein without requiring land resources. Also, microbial protein recovery can be a byproduct of industrial processes, such as biorefineries and breweries, using yeast and algae, and considered less land resource-intensive.[77,78]

Several factors can influence the nutritional composition of microbial proteins, including essential amino acids. These aspects include the source of the nutritional components, the development phase, environmental conditions, and the species of microbes and substrates employed. In a recent study, researchers produced microbial proteins by employing a wide range of microbial species, including *Methylobacterium extorquens*, *Cupriavidus necator*, *Yarrowia lipolytica*, *Komagataella phaffii*, and *Corynebacterium glutamicum*, to develop the microbial proteins. Protein quality from these microbial sources was higher than from soybeans.[79] Bacteria are considered high-quality protein sources due to the potential to provide amino acid profiles that closely correspond to the FAO/WHO reference values.[80] *Chlorella vulgaris* contains around 38% leucine and valine in its protein composition, equivalent to the essential amino acid levels found in protein recovered from *Saccharomyces pastorianus* brewery yeast.[78] A recently published model simulation showed that by 2025, microbial biomass could replace 10−19% of conventional crop-based protein feed, significantly reducing nitrogen leakage, agricultural greenhouse gas emissions, and global cropland area.[81]

Certain challenges are associated with bacterial protein production, such as bacterial proteins exhibiting higher nucleic acid content than microalgae and fungal proteins.[6] This happens during purine catabolism, where guanine and adenine are broken down into uric acid. Uricase enzyme deficiency among humans makes them excrete urate in urine instead of metabolizing it. When the excretion is impaired, excessive accumulation of uric acid can cause conditions such as gout or hyperuricemia.[82,83] Therefore, reducing the nucleic acid content in bacterial protein is important, possibly by applying heat treatment or using methods similar to those used while processing mycoprotein. Bacterial types like *Bacillus cereus* produce toxins that pose some health hazards.[84] Bacteria strains may have no nutritive substances, which, if ingested can be harmful. For example, polyhydroxybutyrate accumulates in the cytoplasm of *Cupriavidus necator* H16 which has been shown to accumulate in organs during studies involving Sprague−Dawley mice.[85] Hence, these potential risks should be evaluated and managed during bacterial protein production. Wastewater becomes a source of bacteria growth, requiring a stringent control system for detecting biological and chemical pollutants. Alloul et al.[86] research showed that cultivation of purple nonsulfur bacteria together with wastewater often contains additional unrelated bacterial populations such as *Arcobacter* spp., *Dysgonomonas* spp., and *Acinetobacter* spp., emphasizing his concern regarding potential quality assurance issues in the bacterial protein production.[86]

Mushrooms. Mushrooms have been placed in a separate kingdom and are not considered animals or plants due to their unique cellular structure.[87] Fresh edible mushrooms have a dry-weight nutritional composition of 84% protein, 3% carbohydrates, 3% fiber, 2% fat, and 8% ash. A research study evaluated the protein composition of 23 types of edible mushrooms, showing protein content ranging from 8.5% to 36.9%, which were substantially higher than those found in cereals, fruits, and vegetables. Trichloma possessed the highest protein content at 36.87%, while tremella had the lowest at 8.46%. Prominent species like *Lentinus edodes*, *Volvariella volucea*, and *Boletus* showed protein compositions ranging from 15.38% to 12.16%.[88,89] Edible mushrooms are considered a functional food and are commonly used as meat substitutes to improve the physical and sensory attributes and nutritional value of meat products. One of the most important examples of functional food applications is mycoprotein, produced by fermentation fungus in liquid media while immersed underneath.[90]

The use of mushroom proteins as nonanimal protein sources is reflected by several challenges documented in the literature.

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Their amino acid profiles may be incomplete and often low in essential amino acids, e.g., methionine or lysine, making mushrooms not a stand-alone protein source unless combined with other proteins for a better balanced amino acid profile.[91,92] The digestibility of mushroom proteins can be less than that of conventional animal proteins, which may influence their nutritional value and bioavailability upon consumption.[93] The umami flavor inherent to mushroom proteins can be attractive for specific applications in meat products but not all food products. Therefore, covering up or modifying this taste to suit its different uses is challenging.[94] Getting high-quality protein from mushrooms affordably is challenging. These methods would yield lower and require more processing, affecting the protein's functional properties and higher costs.[95] Mushroom proteins are difficult to produce at a large scale considering industrial demand scaling because growing mushrooms, extracting proteins, and maintaining final product consistency all require substantial investment and technological advancements.[96] Moreover, the use of mushroom proteins in food products must meet stringent regulatory standards. In various regions, these novel substances could necessitate broad safety testing and approval processes thus delaying market entry.[97]

Jellyfish. It has been demonstrated that marine animals are increasingly being used to extract bioactive compounds and natural defense metabolites.[98] Jellyfish (*Scyphomedusae*) is a valuable source of bioactive compounds due to their distinctive chemical profile.[99] The global fishery that focuses on the collections of edible jellyfish, primarily based in Southeast Asia, generates more than 750,000 tons of jellyfish annually and has markets beyond Asia.[100] The nutritional and therapeutic benefits of jellyfish have been well documented in traditional Chinese medicine, highlighting their potential as a beneficial dietary component. Several species of jellyfish belong to the cnidaria class and may be found in the Mediterranean Sea. These jellyfish have the potential to be profitable resources for industries such as the cosmetics, nutraceuticals, and pharmaceuticals industries. Jellyfish are considered one of Europe's most notable food sources due to their high protein content, particularly collagen.[100] Jellyfish collagen is compatible with human collagen, which extends its possibility of being utilized in various biomedical applications.[101] The calcium-binding protein discovered in jellyfish is believed to improve human brain function and cellular health.[102] Different species of jellyfish and tissues within the same species have been presented with varying protein levels, highlighting their complex biochemical composition.[103] The diverse biotechnological opportunities presented by proteins derived from jellyfish underscore the necessity for further research and application across various fields.

Jellyfish fishery, markets, and consumption in the West are limited because jellyfish products are not in high demand, and there is an absence of jellyfish processing technologies compliant with Western safety standards. The West is mostly unaware of jellyfish, which builds up negative perceptions around them. Therefore, expanding the use of this food will depend upon developing innovative processing techniques and market demand creation that feeds off better knowledge about jellyfish as raw material for food and improved public perception.[13] Jellyfish can also combat fatigue, stimulate blood flow during menstruation, and diminish swelling.[104] Laboratory investigations found that edible jellyfish have collagen, which can be hydrolyzed enzymatically to produce peptides with antihypertensive properties.[105]

Nonetheless, many claims reporting the medicinal values of these organisms are found in nonscientific Chinese publications.[104] Recent European regulations on novel foods (No. 2015/2283) and the biochemical characterization of bioactive properties on some Mediterranean jellyfish species have opened the possibility of classifying Western jellies as "novel food" for human consumption. Nevertheless, it is important to investigate their safety, including possible microbial, chemical, physical, or allergenic hazards due to human intake, before widespread commercialization and consumption in Europe take place.[106] Various nonconventional protein sources with their protein ratio are presented in Table 1.

Table 1. Details on Various Nonconventional Protein Sources and Their Protein Content

source	name	protein content on dry matter basis (%)	ref
microalgae	*Auxenochlorella protothecoides*	61	2
	Arthrospira platensis	60−70	2
	(*Spirulina platensis*)	53.5	198
insects	*Protaetia brevitarsis*	85	49
	locusts	61	55
	damselflies	55	55
plant (leaves)	*Moringa oleifera*	23−43.5	66
	water hyacinth	>50	63
	pumpkin plant	11.75	63
	amaranth	10.7	63
microbial protein	hydrogen-oxidizing bacteria	58	199
	aerobic heterotrophic bacteria	21−49	200
mushroom	*Trichloma*	36.87	89
yeast	*Lentinus edodes*	15.38	201
	Yarrowia lipolytica	45−55	
jellyfish	*Rhopilema esculentum*	53.87	15
	Rhopilema. hispidum	43.80	15
	Rhopilema esculentum	38.12	15

DOWNSTREAM PROCESSING TECHNOLOGIES FOR PROTEINS FROM NONCONVENTIONAL FOOD SOURCES

Ultrasound-Assisted Extraction. Ultrasound-assisted extraction (UAE) is a green extraction method that utilizes sound waves above 20 kHz produced by piezoelectric or magnetostrictive transducers. The efficiency of UAE mainly relies on key parameters such as amplitude, substrate-to-slurry ratio, sonication duration, and sound wave energy intensity.[21,22,107] An acoustic tool, sonotrode, ultrasound probe, or transducer is placed into the liquid medium holding the substance of interest for sound wave introduction. Sound waves propagate in the medium, causing energy transfer and bubble cavitation. Acoustic cavitation constantly forms, grows, and collapses bubbles. Since the medium has significant shear stresses, the collapse of bubbles releases energy, ruptures the cells, and accelerates extraction.[23,24] The UAE can be used alone or in combination with microwaves, alkalis, and enzymes to extract proteins, lipids, polysaccharides, and bioactive compounds.[9,108−113] UAE changes structural and functional attributes, i.e., protein cross-linking, aggregation, foaming,

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emulsification, solubilization, and hydrophobic properties. These changes affect the nutritional properties and functionality of the target proteins.[114] The sonochemical effects of UAE are related to the formation of highly reactive radicals, i.e., hydrogen ($H^{\bullet}$) and hydroxyl ($OH^{\bullet}$), during the sonolysis of water in the medium caused by the extreme conditions generated by acoustic cavitation. These radicals can initiate or participate in chemical reactions, such as oxidation of proteins. UAE application for protein extraction from various protein sources such as edible insects, marine sources, and plant leaves are discussed in Table 2.

Insect proteins are considered a sustainable alternative to conventional animal protein sources, as reported by Gravel et al.[48] They can improve the functional and nutritional attributes of food when used as an ingredient. A study[115] was conducted to evaluate the effect of substituting meat proteins with grasshopper (*Sphenarium purpurascens*) protein and concluded that ultrasound-assisted alkaline extraction (UAAE) enhanced the functional properties of sausages with 10% insect protein substitution, which enabled their usage as an extender in meat products. Moreover, defatting the insect powder can enhance the protein extraction rate. The protein content of Bombay locust (*Patanga succincta* L.) can be increased by up to 78.7% through hexane defatting. Utilizing ultrasound-assisted extraction (UAE) with 60% amplitude and 20 min of sonication time has been found to enhance protein recovery compared to traditional extraction techniques, which required 12 h and did not meet the quality requirements of the food industry.[116] UAE methods to isolate proteins from black soldier flies have significantly improved several protein attributes. This approach has been proven to increase protein digestibility, solubility, and emulsifying capacity, with an extraction efficiency of 80.37%.[117] Furthermore, another study found that proteins from mealworm (*Tenebrio molitor*) extracted with UAE had a high protein content of 60.04%. This yield was achieved with 30 min sonication time and resulted in increased protein solubility, water holding capacity (WHC), oil holding capacity (OHC), and emulsification properties.[118]

Marine organisms contribute to food and health production by providing various beneficial components, particularly proteins. Macroalgae proteins (MPs) have a better amino acid profile than plant proteins, making them rich in essential amino acids with various functional, nutritional, and medicinal properties.[119,120] MPs play a role mainly in the production of single-cell proteins. They can be extracted via UAE from giant kelp (*Macrocystis pyrifera*) with a high protein recovery of 60% via 150 W ultrasonic power for 15 min.[121] Proteins from green algae, particularly *Chlorella vulgaris*, were extracted using ultrasonic-assisted enzyme techniques. Protein recovery rates were from 58% to 82% when UAE was combined with enzyme proteases. However, when the UAE was combined with lysozyme, protein recovery rates varied from 33% to 42%.[122] Moreover, proteins from freshwater green alga (*Chlorella pyrenoidosa*) were extracted using ultrasound-assisted alkaline extraction (UAAE) by following the parameters of 20 min of ultrasonication time, 3% (w/v) alkali concentration, 45 °C temperature, and 360 W ultrasonic power, resulting in a 52.36% protein content.[123]

Compared to the conventional extraction of protein from blue-green algae (*Spirulina platensis*), UAE increased protein digestibility from 16.08% to 33.44% and increased yields of 32.48% to 76.83%. Additionally, it improved the thermal stability, foaming, emulsification, and WHC of proteins.[124] Moreover, collagen was extracted from jellyfish (*Acromitus hardener*) and starfish (*Acromitus rubens*) using UAE with an improved extraction rate.[125,126] Parandi et al.[127] studied extracting protein from mushroom byproducts using a two-stage UAE method. The first phase utilized an ultrasonic frequency of 20 kHz, a temperature below 45 °C, and a power of 400 W. The second step scaled up the process using a 1000 W ultrasonic processor at 24 kHz, which led to an increase of 17% in the extraction efficiency of shiitake mushroom (*Lentinus edodes*) protein.[128] UAE extraction resulted in a protein yield of 82.07 mg/g from moringa (*Moringa oleifera*) leaves using 81 W microwave power, 41 °C extraction temperature, 148 s extraction time, and 91:1 solvent/solid ratio.[129] UAE at 50 °C for 20 min with 450 W/L ultrasonic power density provided the greatest protein content of 30.03% in mulberry leaves, with good *in vitro* digestibility, antioxidant capacity, emulsification, and solubility.[130] Furthermore, jackfruit leaves (*Artocarpus heterophyllus* L) treated with UAE resulted in 96.3 mg/g protein content, with increased foaming and emulsifying capabilities.[131] UAE tends to significantly improve the hempseed protein isolates (HPI) yield by a maximum of 2.1. The increased yield was reported to be higher due to the high shear and mass transfer effects generated by the acoustic cavitation. At neutral pH (7.0), the protein's highest solubility is 53%, achieved under 26.8 W/cm^2 for 5 min of ultrasound, which is about twice that of conventionally extracted HPI.[132]

High-Pressure Extraction. High-pressure-assisted extraction (HPE) is environmentally safe for disrupting biological structures such as cell walls, membranes, and organelles. Its application improves mass transfer dynamics, increases extraction efficiency, shortens extraction time, and minimizes solvent consumption to extract proteins.[25] HPE induces the breaking of ionic bonds that tend to decrease in volume because of electrostriction forces acting on water. Hence, the application of HPE can alter the existing structure of the biomolecule of the cell. The change in structure is responsible for the denaturation of proteins by breaking the hydrophobic interaction and salt bridges that ultimately collapse the organelle's cell walls and cell membranes. The extraction rate of proteins using HPE mainly depends on the nature and volume of solvent used, applied pressure, extraction time, number of extraction cycles run, and temperature.[133]

High-pressure extraction (HPE) techniques are increasingly used to isolate and extract nonconventional proteins, particularly from plant sources, enhancing efficiency and yielding higher protein recovery while addressing challenges posed by the complexity of biological sources. HPE can alter protein solubility and increase extraction solvent penetration into cells by altering matrix structure. It is an important breakthrough in food science and biotechnology for cellular content extraction.[26] Applying uniform isostatic pressure (100−1000 MPa) using the high hydrostatic pressure (HHP) technique induces modifications to the structure of cellular and protein components. Initially employed to eradicate microbes in food processing, HHP evolved its application to extract phenolic compounds and proteins. The effect of HHP on protein properties varies according to the specific protein and its environment, ultimately improving digestion and processing properties. Proper optimization of process parameters is crucial for efficiently releasing bioactive peptides through enzymatic treatments.[134]

Table 2. Downstream Processing Techniques of Different Protein Sources along with Method, Parameters, Protein Purity and Recovery, Limitation, and Results

protein source	method	parameters	protein purity and recovery	limitations	results	references
Chlorella vulgaris and *Neochloris oleoabundans*	Pulsed Electric Field (PEF)	1−40 pulses, 0.05−5 ms pulses, 7.5−30 kV cm^{-1}, 0.05−150 kWh kg DW^{-1}	13% of proteins released	The electroporation effect proved insufficient to release intracellular proteins significantly.	Both algal species exhibit the liberation of ions, signifying the permeabilization of the algal cell due to pulsed electric field (PEF) treatment.	154
Sesame cake	Pulsed electric field (PEF), ultrasound, and high voltage discharge (HVD)	Energy inputs between 0 and 291 kJ/kg (0−700 pulses)	–	–	PEF and HVD minimize ethanol reliance as a solvent for extracting phenolic compounds, eliminating the necessity for elevated temperatures to enhance diffusion	156
Olive kernels (*Olea europaea*)	PEF and HVD	HVD energy input (0−109 kJ/kg), pH (2.5−12), and ethanol (0−50%)	Protein recovery ranges from 0.16 mg/mL to 0.48 mg/mL	Elevated temperatures were required to enhance diffusion	HVD treatment outperformed ultrasound and PEF in terms of energy efficiency and extraction time for phenolic compounds and proteins	155
Beer waste Brewing yeasts	PEF	Electric field strength (10 to 50 kV/cm), pulse numbers (2 to 16 μs)	Protein yield 2.788 ± 0.014%	–	Improved permeability of yeast cells demonstrated promise in extracting protein from waste beer yeast, offering potential benefits to the food and agriculture industry.	158
Microalga *Haematococcus pluvialis*	PEF	field strength of 1 kV/ cm	46% of the total amount of proteins was recovered	Proteins of no interest were also isolated	Application of this biocompatible electro-extraction method in biotechnological processes can efficiently produce 25 μg of proteins per mL of culture medium.	159
Rice bran	Microwave-assisted extraction (MAE)	frequency of 50−60 Hz used	Protein yield: 15.68% Extraction time: 2 min	–	Higher protein yield in a short time, no protein damage	202
Soy okra	MAE	Multimode microwave reactor 2.45 GHz with a maximum delivered power of 1000 W variable in 10 W increments	Extraction yield: 0.23% Protein content 18.5%	Higher processing temperatures can alter the functional attributes of the proteins.	The solubility and digestibility of proteins in extracted soymilk exhibited significant improvement.	203
Jackfruit leaves	MAE	2, 3, or 4 min (applying pulses of 30 s) at 1200 W	Protein content: 9.56%	The optimum temperature for protein extraction is 60.1 °C, which can lead to the denaturation of proteins Resulted in a loss of solubility	Minimum waste production	204
Coffee silver skin	MAE	Microwave level of 434.7 W	Protein yield: 43.53%	–	Recovery yield significantly increased 5.8-fold and 7-fold	205
Bombay Locust	UAE	10, 20, and 30 min, Power: 750 W Frequency: 20 kHz Amplitude: 40, 60 or 80%, 25−35 °C	Protein recovery was highest (15.57%) at 60% amplitude for 20 min	Protein solubility was only improved at pH higher than 2	Enhanced protein yield and functional and antioxidative properties in comparison to the conventional method	116
Black soldier fly	UAAE	1 h, Power: 4.57 W/cm^3, 25 °C, 2.45% NaOH	80.75% protein purity	Reduced protein foaming properties	Improved emulsification property and protein solubility	117
Yellow mealworm (*Tenebrio molitor*)	UAE	Time: 10, 20, 30, 40, 50 min, Power: 4 W/cm^3, Frequency: 28 kHz at 25 °C	Maximum protein yield was 60.04% obtained at 30 min.	–	Increased protein solubility, WHC, OHC, and emulsification properties	118
Jellyfish	UAE	Time: 0, 20, 40, 60, 80, and 100 min Power: 220 W Frequency: 40 kHz Temperature: 45−54 °C	The highest soluble protein content (0.528%) was obtained with 100 min of sonication time.	Protein gel strength increased with sonication time but reduced at 100 min sonication time due to structural changes gelatin quality was inferior to bovine and fish gelatin	UAE enhanced jellyfish protein desalination and extraction rate	125
Fresh common starfish (*A. rubens*)	High shear mechanical homogenization combined with UAE	Amplitude: 75% Time: 10 min Temperature: 25 °C	83.18 ± 1.74% protein content	Collagen yield was similar to the conventional method	Improved protein and collagen extraction rate	126
Mollusks (abalone)	UAE	Time: 52 min, Power: 428 W, 14 g/kg (NaOH)	64.89% protein yield	–	UAE generated abalone derived iron chelating peptides	206
Giant Kelp (*Macrocystis pyrifera*)	UAE	Time: 5, 10, and 15 min, Power: 50, 100, and 150 W, Frequency: 40 kHz at 25 °C	The highest protein recovery, 60%, was obtained via 150 W and 15 min.	–	Enhanced protein extraction and recovery	121

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Table 2. continued

protein source	method	parameters	protein purity and recovery	limitations	results	references
Chlorella vulgaris (*C. vulgaris*)	UAE with enzyme	Time: 10 min Frequency: 35−130 kHz, at 50 °C with protease (0.1% v/v)	82.1 ± 1.1% protein recovery		UAE with protease enzyme enhanced protein recovery than without using any enzyme	169
Chlorella pyrenoidosa	UAAE	Time: 20 min, Power: 360 W, at 45 °C with alkali 3%	52.36% protein yield	–	Enhanced yield antioxidant and thermal properties	123
Microalgae (*Spirulina platensis*)	UAE	Time: 10, 20, 30, 40, 50 min, Power: 4.57 W/cm^3, Amplitude: 20, 40, 60, 80, 100%, Duty cycle: 20, 40, 60, 80, 100% and 2.45% NaOH	76.83% of the highest protein yield was obtained at 30 min, with 60% and 80% amplitude	UAE enhanced blue color intensity of protein powder	Increased WHC, foaming, and emulsification capacity	124
Moringa leaves	MAE	Time: 148 s Ultrasound Power: 300 W, Frequency: 25 kHz, Amplitude: 40, 60 or 80%, Microwave power: 81 W at 41 °C	82.07 mg/g protein yield	–	Exhibited significantly higher extraction efficiency than the conventional solvent extraction method, which was 68.99 mg/g	129
Mulberry leaves	UAE	Time: 2.5, 5, 7.5, 10, 12.5, 15, 17.5, and 20 min, Power densities: 150, 300, and 450 W/L and Temperature: 30, 40, and 50 °C	Maximum protein yield was 30.03% with 450 W/L power at 50 °C for 20 min.	–	Caused structural changes in protein and significantly enhanced the *in vitro* digestibility, antioxidant, and emulsifying property	130
Jack fruit leaves	UAAE	Time: 20 min Frequency: 42 kHz, Alkali concentration: 0.5, 1, or 2 M NaCl	0.5 M NaCl yields the highest protein content of 96.3 mg/g	–	Improved emulsifying and foaming properties	131

High-pressure homogenization (HPH) improved pumpkin press cake aqueous extraction by improving protein yield (61.92% at AE+100 MPa), solubility (30.21% for 100 MPa HPH+AE), and techno-functional properties, including emulsification and foaming capacity. HPH treatments tend to decrease the protein denaturation temperature and particle size, with 100 MPa AE+HPH being proven more effective in this aspect.[135] For plant protein separation, alkaline extraction (AE) and isoelectric precipitation replace solvent-based techniques that offer low protein yield and degradation. Combined with high-pressure homogenization, AE significantly increases yield and protein quality, achieving yield levels as high as 61.92 ± 1.06% compared to the 45.99 ± 0.72% obtained by traditional techniques. This synergistic approach improves protein solubility and foaming, emulsification, color, and thermal stability properties.[135]

HPH pretreatment increased carbohydrate and protein recovery from freshwater green alga (*Chlorella pyrenoidosa*) after a four-pass treatment at 800 bar and a 24 h incubation at 40 °C. Using ethanolic and aqueous extractions with biomass processed at 800 bar, 4 passes produced water extracts with high protein content and chlorophyll separation.[136] Research on pressure-induced protein denaturation has revealed reversible and irreversible changes, resulting in aggregation or gelation of proteins. Oligomeric protein structures have been found to denature at pressures lower than 200 MPa.[137] However, there is limited research on the chemical stability of proteins under high hydrostatic pressure (HHP) and oxidation effects reported in the literature. Protein oxidation in pork batters treated with HHP increases nonheme iron levels through carbonylation and decreases sulfhydryl groups. Despite changes in protein contents, the end product's nutritional and microbiological quality has not been compromised.[137]

Dry Fractionation Extraction of Proteins from Nonconventional Protein Sources. Different extraction methods for protein extraction from novel sources, such as water, organic solvents, and enzymes, present a varying level of effectiveness depending on the cell composition and the solvent used. However, the frequent use of chemical solvents, which are not environmentally friendly or safe, is still widespread.[17] To improve the recovery rate of insect-based materials after extraction and chitin removal, it has been recommended to use pretreatments such as blanching, freezing, and thawing in response to the demand for cleaner extraction procedures. In combination with pretreatments, dry fractionation is an effective technique for developing protein-rich concentrates from insects while decreasing the chitin content.[138]

A study examined the effects of different postharvest treatments, drying, and defatting methods on the physicochemical characteristics of mealworm larvae and the factors influencing their disintegration during roller milling. The considerable changes in color, size, density, and hardness influenced the fractionation of protein-enriched fractions.[139] Berghout, Boom, and van der Goot[140] conducted a study on extracting eco-friendly lupin seed protein without employing organic solvents. They discovered that adjusting extraction temperatures enabled the development of protein isolates with low oil content while maintaining their functionality similar to those extracted with conventional methods. The concentration achieved using ultrafiltration was 0.25 gmL^{-1}, which is ideal for high-protein uses. Another study explored the application of

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dry milling for pea protein to maintain its original functionality while minimizing resource consumption. Various milling methods resulted in different flour quality, and excessive milling damaged the integrity of proteins.[141]

Aye and Stevens[142] studied the process of isolating protein and chitin from black tiger prawn shells using dry fractionation. The shells were dried at 55 °C for 24 h until they reached a 3−5% moisture level, then crushed into 2−5 mm fragments. A 0.85 mm filter separated the components, raising the protein content from 28.7% to 33.0%, with 57.1% of the shells passing through the sieve. Purschke et al.[143] explored that blanching, drying, and defatting affected yellow mealworm larvae fractionation and protein properties. Freeze-drying and partial defatting with supercritical CO_2 extraction resulted in smaller particles (<500 μm) and improved crude protein content up to 66% by eliminating fat content. Sipponen et al.[144] analyzed house crickets and mealworms using supercritical CO_2 extraction and milling techniques. The fractions differed in amino acid composition, solubility, and sensory properties, with mealworms having a slight protein content increase. The protein and chitin-rich coarse fraction had lower solubility and distinct sensory properties than finer protein powders.[144]

Ravi et al.[145] suggested an industrial fractionation method that includes electrostatic protein extraction from black soldier fly larvae (*Hermetia illucens*). This procedure involves selecting suitable electrostatic separation equipment and adjusting various factors such as particle size, air flow rate, voltage, angle of charge plate(s), size, charging tube material, design, and treatment duration. Optimization of these parameters is crucial for increasing protein recovery efficiency and maintaining final product quality. Various studies[146−149] have used comparable optimization methods for lupin, navy beans, and other protein sources. Past research has shown that combining cascade milling and fractionation with recycling specific protein fractions can significantly increase the quantity and quality of final proteins obtained.[149,150]

Furthermore, combining various dry fractionation techniques such as air classification, sieving, electrostatic separation, and magnetic separation, the overall product yield and purity of extracted proteins can be improved.[151] Another study also reported the effect of dry fractionation on obtaining a dry protein-rich fraction using a supercritical CO_2 extraction from milled rice bran. The results found that nonmilled raw material fractionated by air classification yielded 19.7% of protein; however, the one-step air classification increased the protein fraction from 18.5% to 25.7%.[152] The dry fractionation was also employed by involving milling and air classification of mung bean flour, resulting in protein concentrates with a fine fraction with a protein content of 62.2 g/100 g, representing a 2-fold increase in protein content compared to the original flour.[153]

Pulsed Electric Field and High Voltage Electrical Discharge Extraction. Pulsed Electric Field (PEF) and High Voltage Electrical Discharge (HVD) are popular nonthermal extraction technologies that use high voltage to generate an electric field that produces electroporation, allowing for the extraction of intracellular components from cells.[27] PEF is a technique that uses high-voltage pulses to create reversible or irreversible disintegration in cell membranes, enabling important substances such as proteins and polyphenols to be recovered.[28] Similarly, high-voltage electrical discharge in a liquid medium creates plasma channels for extraction. Both procedures have advantages over heat-assisted extraction methods, as they preserve thermolabile components such as proteins and improve extract quality during processing and storage. Their minimal energy consumption is consistent with environmentally sustainable extraction practices. Recent studies have shown that PEF and HVD are successful at extracting proteins and other chemicals from a variety of sources, including olive kernels, sesame cake, and microalgae.[154−156] While HVD effectively extracted phenolic chemicals and proteins, PEF showed promise in modifying protein solubility and functional characteristics. For example, PEF treatment of canola seeds improved protein solubility and functional properties, whereas peptides produced from pine nut proteins showed changes in secondary structure and antioxidant activity.[157] PEF has been used to extract protein from various sources, including beer waste yeast,[158] the microalga (*Haematococcus pluvialis*),[159] and plant-based protein sources.[19] These experiments revealed PEF's potential to improve protein extraction efficiency and yield. Furthermore, PEF is a biocompatible approach to protein extraction because it does not affect the source material's viability.[159] These data indicate that PEF is a potentially efficient protein recovery and extraction method for nonconventional protein sources.

Microwave-Assisted Extraction. Microwave-assisted extraction (MAE) uses electromagnetic waves (300 MHz to 300 GHz) to disrupt the cells.[19] These waves are absorbed and switched into thermal energy, which heats the cell's moisture. High pressure on cell walls improves porosity and facilitates chemical extraction.[160] During MAE, the cellular matrix gets exposed to microwave radiation for a short period. As a result, the temperature of the matrix rises. The increase in temperature causes evaporation of the intracellular water, induces pressure changes, and ultimately leads to cell disruption.

Sample solubility, solid−liquid ratio, extraction duration and temperature, microwave power, system agitation, dielectric constant, and dissipation factor are all important MAE factors to optimize and scale up the process. Kumar et al.[161] demonstrated the potential of this technique for addressing modern food processing concerns such as cost-effectiveness and sustainability. Chew et al.[18] provided specific examples of how MAE successfully recovered protein from microalgae and archival tissue. Microwave-assisted three-phase partitioning is an efficient, cost-effective, and environmentally friendly method for extracting bioactive chemicals from microalgae.[18] A study reported using MAE to extract protein from microalgae (*Nannochloropsis oceanica*) and (*Nannochloropsis gaditana*) using a closed vessel for exposure to microwaves. For this purpose, the microwave oven was equipped with a time controller to assess the duration of on−off irradiation cycles at 700 W energy with a frequency of 2450 MHz. The results reported that the MAE technique succeeded in extracting about 65.06% of the total protein from both species and proved a significantly higher yield than the conventional protein extraction.[162]

Cold Atmospheric Pressure Plasma. Pretreatment with cold atmospheric pressure plasma (CAPP) enhances protein extraction. In the gas phase, CAPP's high-energy electrons and ions generate reactive species such as singlet oxygen, superoxide radicals, and nitric oxide. These reactive species increase oxidative stress, which disrupts the cells.[29] Lipid peroxidation alters the lipid bilayer structure, deteriorating the cell membrane's integrity and increasing cell permeability.

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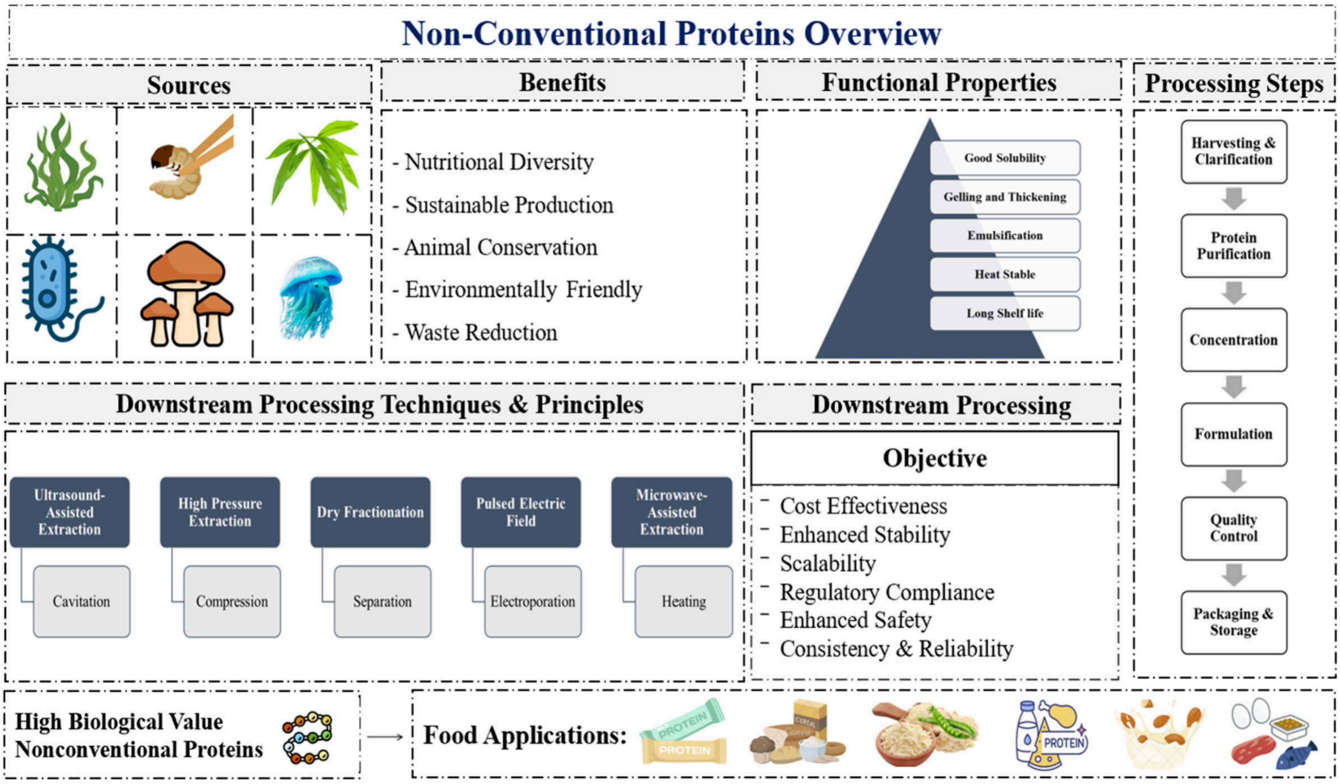

Figure 3. Pictorial representation of nonconventional protein sources, their benefits, and nonthermal processing, including principles and potential applications.

CAPP produces electric solid fields that cause electroporation, forming pores in the lipid bilayer.[30] These pores allow ions and chemicals to pass through, permeabilizing membranes and releasing biological components. CAPP-induced local heating can denature proteins, exposing their hydrophobic regions and facilitating protein extraction.[163]

CAPP has emerged as a viable nonthermal technique for altering proteins from novel sources, especially plant-based materials. Different studies[107,164] highlighted the potential of CAPP to improve protein quality, functionality, and allergenicity reduction. Bußler[138] further presented the application of CAPP in modifying secondary metabolite profiles in plants and the techno-functionality of flours and proteins from peas, demonstrating CAPP's ability to enhance pea flour's water and fat-binding ability. Another study examined how cold atmospheric air plasma affects hemoglobin, pork gelatin, and nonconventional bovine lung functional protein. Protein native structure and nature affected proteins' functional, rheological, and gelling properties. Plasma-protein interactions are specific, highlighting the need for protein research under different plasma operating conditions.[165] A study reported the effects of CAP treatment on whey protein isolation for 1 to 60 min. The study's results reported a small but significant increase in the surface hydrophobicity index up to 15 min of treatment, attributed to slight oxidation of the WPI. This oxidation modifies the side chains of amino acid residues, ultimately increasing carbonyl content and surface hydrophobicity.

Additionally, the strong oxidation of cysteine along with protein aggregation, resulted in a loss of free SH groups. While foaming improved within the first 15 min of exposure, it significantly decreased after this period, although foam stability increased.[164] The pictorial view of various benefits and nonthermal processing techniques of nonconventional protein sources is presented in Figure 3.

Functional Properties of Nonconventional Proteins and Applications in Food Products. Studies on various protein sources, including edible insects, jellyfish, microalgae, mushrooms (*Pleurotus ostreatus*), plant leaves, and microbial proteins, provide thorough insights into their functional characteristics. Table 3 highlights the functional attributes and potential dietary uses of nonconventional sources. Techno-functional properties of proteins are important for formulation or ingredient substitution during food processing. The most studied functional properties are solubility, emulsion capacity and stability, foamability, gel formation, water-holding capacity, and oil-holding capacity.[166]

A study reported that desalted dried jellyfish (*Lobonema smithii*) produced type A gelatin by acid hydrolysis at pH 1 by maintaining water extraction at 45 °C, 60 °C, or 75 °C for 6 or 12 h. The highest gel strength (118 g) of type A jellyfish gelatin was obtained after a 6 h extraction at 45 °C. However, gelatin produced at 75 °C for 6 h exhibited the highest emulsion stability index (37.72%). Results showed that Type A jellyfish gelatin could be produced with improved gel-forming, emulsifying, and foaming properties.[167] Boiling salted jellyfish (*Rhizostoma pulmo*) without water turns it greyish, thickens it, causes foam flakes to appear, and removes its specific smell, enabling it to be used with other food products as an ingredient.[168] Moreover, salted jellyfish gelatin samples resulted in a soft gel on hydrolysis. Gelatin extracted from jellyfish in just 12 h yielded a high gel strength of 323.74 ± 7.02 g and viscosity of 7.73 cP, comparable to other commercial gelatins.[169] A potential zeta study also reported

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Table 3. Functional properties of novel protein sources and their food applications

parameter	sources	results	factors	food applications	ref
Color	Jellyfish	Brown color protein gel	Salted jellyfish and dried desalted jellyfish gel were dark brown; a freshly caught jellyfish exhibited translucent gel.	Used in colored food and animal feed as a gelling agent	169
Color	Black soldier fly (*Hermetia illucens*)	Brown or dark in color with an L value of 41.58	Extraction temperature maintained at 40 °C	Used in colored baked products	207
Oil absorption capacity (OAC)	(*Hermetia illucens*)	OAC of proteins exhibited was 2.70 and 3.40 (g/g) for the optimized protocol (OP) and modified extract (MP)	OP extract presented a lower total hydrophobic residue than proteins obtained through MP extract.	Use in foods where the physical entrapment of oil/fat is required in ground meat products, doughnuts, and baked products.	207
Emulsion activity	(*Spirulina platensis*) protein extract	Presented optimum emulsion activity of (22.41%) with olive oil	Oil extraction is required at optimum concentrating	Useful to enhance the flavor of oil-based food products	208
WHC	*Arthrospira platensis*	Optimum WHC with the value of 2.11 ± 0.13	Varying amino acid profiles alter the WHC	High WHC values contribute to maintaining moisture in baked products	209
OHC	*Arthrospira platensis*	Optimum OHC with the value of 2.96 ± 0.23 g	Varying amino acid profiles alter the OHC	High OHC values can improve the flavor and palatability of meat analogs	209
Emulsification capacity and solubility	Migratory locust (*Locusta migratoria*)	Exhibited good emulsification stability at pH 5 and maximum solubility (100%) at pH 9	Changes in optimum pH and temperature during processing alter the functional attributes.	Useful as an emulsifying ingredient in emulsified food products	210
Gelling and foaming attributes	Black cricket (*Gryllus assimilis*) protein isolate	During processing, the absence of NaCl led to an increase in the surface hydrophobicity of protein isolates; the heat treatment at 90 °C for 15 min resulted in gelling	Processing variables, including heat treatments (65, 75, 85, 90, and 95 °C for 15 min) at variable ionic strength (from 0 up to 0.5 M of NaCl) affected the functional attributes	Useful as a food ingredient in particular gel-based foods	211
Foaming	Black soldier fly larvae (*Hermetia illucens*) proteins	Optimum foam stability was obtained at 85 °C with 15 min of treatment	Variable temperatures during processing	The 61.1% of protein can be used in food fortification system	212
Food Palatability	Shiitake mushroom glutamate	Umami taste induced by the glutamic acid or glutamate present in the mushroom	Glutamate must not bind to other amino acids in a protein chain.	Increased the palatability of meaty foods by enhancing the umami flavor	213
Texture	Mushroom (*Coprinus comatus*) protein powder	Improved the texture, chewability, and springiness of plant-based protein snack bars	Concentrations concerning other available proteins	It can be used in conjugation with other proteins in the formulation of plant-based snacks and cereals	214
Texture, WHC, and OHC	King oyster and phoenix mushroom	On rehydration and cooking, it provided a minced meat-like appearance and texture.	Exhibited inferior water and oil holding capacities	Useful in the production of value-added meat products	215
Solubility and binding attributes	Perilla seed meal protein	Increased solubility of a meal from 69.62 to 83.10%	Chemical modifications are required to increase the protein solubility	Used as an emulsifier to stabilize the carotenes (bioactive pigment) in a food emulsion system	216
Foaming and stability	Grass pea proteins	Increase the foaming of protein and sugar conjugate by 45.17 % and 37.17 % produced during Maillard reaction; improved the solubility by 98.88 %	Tend to decrease the lightness of the samples by 24.75 % and alter the color profile.	Useful as a foaming agent in various food products with dark color	217
Encapsulation	Sesame protein hydrolysate	Increased the retention of anthocyanins for 90 days at 35 °C by (93.13%) within sesame protein and gum Arabic conjugate	Temperature control is required during the Maillard reaction; otherwise, the color will be altered.	Act as effective wall material and carrier of anthocyanins in food systems	127

that jellyfish collagen type II was stable up to pH 9, and denaturation started at a higher pH.[170]

The solubility of proteins in edible insects depends on the pH level, with the lowest solubility occurring at the isoelectric point. This point typically falls near pH 4 for mealworms and black soldier flies.[171] Cricket powder with 66% protein (dry weight) showed WHC of 1.76 g and OHC of 1.42 g. These commercial powders were obtained by roasting the whole insects at 107 °C and grinding them into powder.[172] A study found that whole yellow mealworm powders treated with freeze-drying and grinding had slightly lower foaming properties that varied among insect species. As a result, roasted and ground mealworm powder was identified as nonfoaming, while cricket powder had higher foaming stability (86%), WHC of 1.29 g, and OHC of 1.71 g. However, concentrating the cricket protein fraction through alkaline extraction and precipitation at the isoelectric point resulted in higher WHC and OHC levels of 3.95 and 2.74 g, respectively.[173] Protein composition can also affect foaming capacity and stability. The protein fraction of mealworm (*Tenebrio molitor*) soluble in salt can produce more foam than the fraction soluble in water.[174] A recent study suggested that the emulsifying properties of proteins from larvae (*Protaetia brevitarsis*) are affected by the solvent used for fat extraction. As a result, hexane showed the highest functional properties, followed by ethanol.[175] A study showed that the emulsifying activity of migratory locust hydrolysates increases at pH 7. Blanching and subsequent fermentation of full-fat and defatted mealworm powders impair the surface activity of the proteins.[176] Heating yellow mealworm protein extract at 95 °C for 60 min increased surface hydrophobicity and aggregation, resulting in a stronger gel than using lower temperature and shorter time.[177] A low concentration of IPs, such as yellow mealworm powder or house cricket flour, can improve the hardness and gumminess of meat products. On the other hand, increasing the concentration of IPs from 10 to 25% can negatively affect the texture of the meat, making it chewier due to increased elasticity caused by the formation of a protein network.[178,179]

Application in Meat Products. Edible insects are becoming a sustainable protein source for meat products due to their low space requirements for growth and associated low environmental impact. Insect-based meat emulsions, especially those with yellow mealworm flour and house cricket, contain 55% protein. Replacing 10% of lean meat with house cricket flour, protein content and micronutrients like potassium, phosphorus, and magnesium are increased without impacting the texture and cooking yield of the final product.[166] Microbial safety and sensory qualities must be considered when adding house cricket flour to emulsified meat products for commercial applications.[180]

The meat industry uses mushrooms to provide taste, carbohydrates, protein, essential amino acids, fiber, and bioactive compounds. Several studies have also examined mushroom proteins in meat products. Banerjee et al.[181] observed that using enoki mushroom stem waste enhanced goat meat nuggets' physicochemical properties, oxidative stability, and shelf stability. Similarly, Kurt and Gençcelep[182] found that adding mushroom powder to meat emulsions enhanced their emulsion stability and textural characteristics. Torres-Martínez et al.[183] emphasized the potential of *Pleurotus* spp. as a source of proteins, bioactive substances, and antioxidants for meat products. Additional investigation is required to address the current challenges in developing and using mushroom proteins in food products, as Yang et al.[184] proposed. Winter mushrooms can be used as phosphate substitutes in emulsion-type sausages to improve texture and provide antioxidative characteristics without negatively affecting the sensory profile of the product. Incorporating microalgae, Chlorella, edible insects, and mushrooms in meat products meets the need for alternative protein sources and provides nutritional and functional advantages to the food sector.

Application in Bakery Products. Insect proteins improve the protein and fat content of muffins, cookies, and biscuits. Consumer acceptance tests, especially in developed countries, allow adding 10% insect protein powder to bread and up to 5% to cookies, biscuits, and muffins. Future research should compare the nutritional value of insect proteins and processing costs to incorporate insect proteins in bakery products.[185] Termites are preferred among edible insects and are integrated into recipes to substitute various flours at 5% to 25%.[37] Despite sensory evaluation showing no significant differences from control samples, consumer acceptability remains limited. Edible insects offer better nutrition and digestion than plant-based proteins but face challenges regarding consumer acceptance.[185]

Incorporating 5.0−10.0% by weight of *Dunaliella salina* microalgae into dough improved sensory and physical-chemical attributes of the final product.[185] Incorporating microalgae biomass into wheat crackers boosts their antioxidant activity and protein content, making them a certified source of protein. Adding microalga powder to gluten-free bread raises the fat and protein content of the final product.[37] *Chlorella vulgaris* microalgae, rich in proteins, can replace animal fats and proteins in bread products without affecting taste or structure.[37] Substituting wheat flour with jellyfish in crackers increases protein and fat, making them a more nutritious and economical protein source.[185] Crude jellyfish gelatin reduced staleness in frozen tortilla bread by maintaining moisture content, water activity, tensile strength, and elasticity, improving taste, texture, and overall acceptability, while some changes in color preferences were observed.[186]

Incorporating *Agaricus bisporus* mushroom powder into cake recipes increased the protein, fat, ash, and fiber content. The higher the concentration of mushroom powder (from 5% to 15%), the higher the increase in these nutrients, while sugar levels decreased by.[187] Improvements in color, flavor, texture, and overall acceptability were highest when 10% mushroom powder replaced wheat flour. Egg-free cakes with 10% mushroom powder were more nutritious without compromising taste.[188] *Pleurotus eryngii* mushroom flour improved wheat flour mixing and rheology of the cookie dough. 15% mushroom flour enhanced water absorption and peak viscosity but decreased dough stability. With 15% mushroom flour, dough functioning improved by lowering gelatinization temperature and shear elasticity.[189]

Mushroom powder with higher protein content developed a superior gluten network, producing optimum elasticity in bakery products, pasta, and noodles.[190] A study found that mushroom flour had a lower water-holding capacity (WHC) and swelling power at 2.4 g/mL and 2.9 g/mL, respectively, while oyster mushroom flour exhibited the lowest bulk density at 0.8 g/mL.[191] However, the digestibility of mushrooms was reported in the range of 60 to 70%. Furthermore, the digestibility of proteins can be improved by removing food

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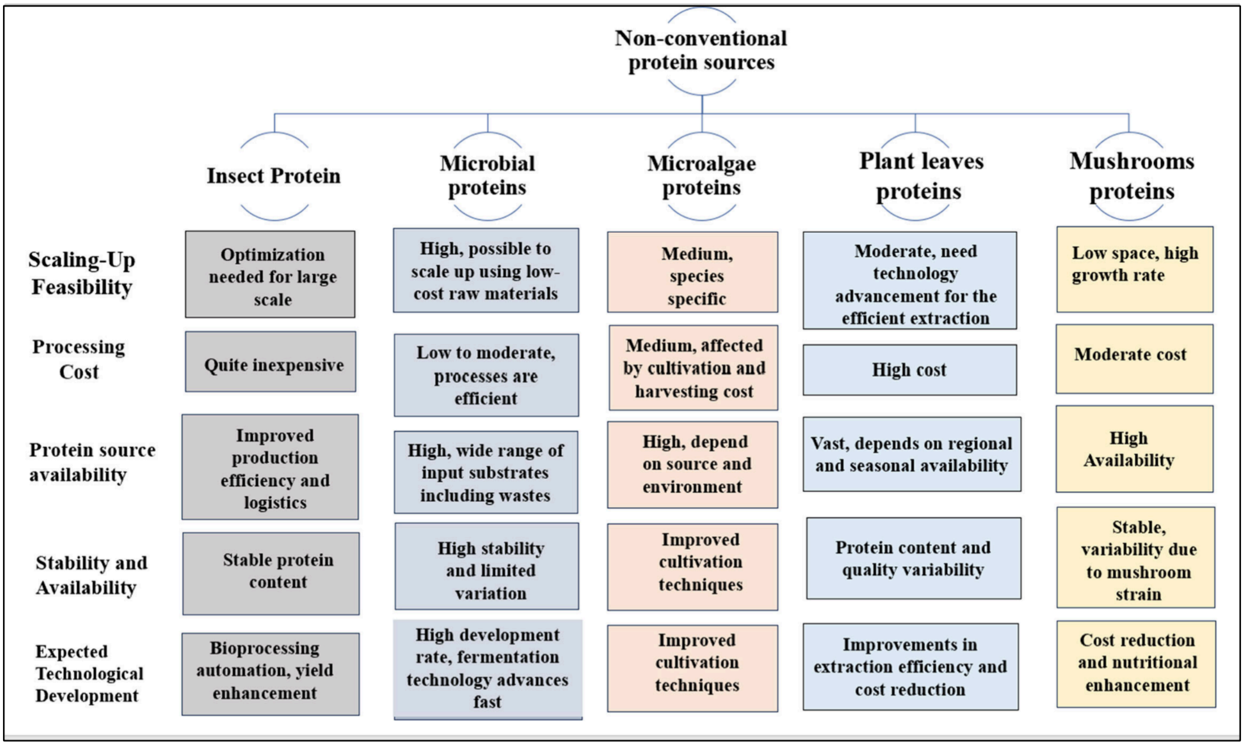

Figure 4. Detailed summary representing various perspectives related to the bioprocessing of nonconventional proteins.

components during digestion or extraction that impede hydrolysis, such as fiber, reducing sugars, and residual lipids.[192]

Application in Cereal Food Products. Pasta is a popular and convenient food; thus, alternative raw materials for pasta manufacturing have been studied thoroughly. With their unique chemical composition and amino acid profile, insect flours may improve pasta nutrition, especially for those without gluten who seek alternative protein sources.[193] Romano et al.[194] examined the effect of cricket powder on dry pasta, observing alterations in cooking weight, color, and hardness. The protein-enriched pasta was well-received by consumers despite changes in flavor, demonstrating its potential as a feasible option. Research indicates that pretreated mealworm larvae and silkworm pupae can be used as innovative protein components in sausages, and insect powders can be used in cereal-based products for protein fortification.[195] However, It is important to conduct comprehensive research to assess the impact of adding insect powders to bread on its technological, nutritional, microbiological, and sensory attributes.[193]

Batista et al.[196] studied microalgae biomass in crackers as a partial substitute for wheat flour to provide a new, nutritious snack alternative. The study examined microalgae's biochemical content, antioxidant capacity, and in vitro digestibility, revealing their potential as a healthy dietary element. Xie et al.[197] investigated the quality of mealworm-enriched, soft, wheat-leavened crunchy snacks. The study evaluated technical, microbiological, nutritional, and sensory characteristics to enhance the processing of insect-based food and the quality of leavened food. It suggests incorporating insect flours, edible insects, and microalgae into cereal-based foods to enhance their nutritional quality and sustainability in the food industry.

■ CHALLENGES AND FUTURE PERSPECTIVE

This review extensively presents various nonconventional proteins derived from insects, fungi, microalgae, jellyfish, and plant leaves, each with distinctive chemical compositions and functional properties. These proteins are increasingly considered sustainable substitutes for animal-derived traditional proteins since they perform functions like emulsifying, foaming, and gelling, which are important in various food applications. However, with the increasing number of studies on nonconventional proteins, there are still several gaps in our understanding. It is pointed out that more research is needed to optimize the functionality and processing by incorporating these proteins into different food systems and developing novel food products that amalgamate nonconventional protein sources with conventional ones. Relevant bioprocessing aspects of nonconventional protein sources, such as scaling-up feasibility, processing cost, protein source availability, stability and variability, and expected technological development, are highlighted in Figure 4.

The production of nonconventional proteins also presents several opportunities and challenges. However, scaling up the protein production process also brings significant technological challenges, such as consistently optimizing the production process to produce better products at lower costs. This could advance the process of producing unconventional proteins that are sustainable and scalable. The expense involved in producing proteins plays a role in determining their success in the market. Existing techniques like fermentation and extraction tend to be costly due to the requirement for specialized machinery, energy consumption, and intricate processing procedures.

Additionally, the production costs of these proteins must remain competitive with protein sources to encourage widespread acceptance. Advancements in processing technologies, such as extraction techniques and integrated bioprocessing systems, are necessary for cost reduction and the commercial viability of these proteins. Insect farming and algae cultivation are accessible protein sources due to their

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minimal resource requirements. On the other hand, some sources, like fungi, may have limited availability due to growth conditions. The sustainability of these sources is a benefit since many unconventional proteins can be produced using natural resources compared to traditional livestock farming. This sustainable element supports food security objectives because these sources are more resistant to climate change and resource shortages.

Nonconventional proteins' chemical composition and functional properties remain a significant concern due to their stability and variability. Maintaining these proteins' nutritional and functional qualities during processing and storage is crucial and requires stability in these properties. Protein content and quality can vary due to different methods, environmental factors, and genetic variability. Consistent product quality and consumer acceptance depend on addressing these factors. Various methods must be implemented to integrate nonconventional proteins into the mainstream food system, including product development, technology innovation, consumer awareness, regulatory compliance, and economic strategies. Debittering is required to enhance palatability and acceptance by consumers of the proteins mentioned above, which entails physical and chemical techniques and sensory masking applications. Some common ways of debittering are encapsulation, blending with standard protein, processing aspects like fermentation and extrusion, and advanced ones that include 3D printing or cell culture. These allow for incorporating alternative proteins into daily diets, addressing global food security, health, and environmental sustainability concerns. There has been remarkable progress, as evidenced by this review. This entails addressing current challenges and envisioning future research pathways to develop sustainable, protein-rich foods to meet the growing global demand. The above concerns underline the significant potential and problems associated with commercializing novel proteins. Addressing these challenges through continuous research, technological advancements, and industry collaboration will be essential to fully leveraging sustainable protein sources.

■ CONCLUSION

The nonconventional protein sources have received increased attention in recent years. These protein sources are studied for extraction, composition, functional properties, and nutrition. Each source has advantages and constraints in terms of nutrition and functional attributes. Despite developments, challenges remain for applying protein from unconventional sources, including requirements to enhance further proteins' nutritional, sensory, and ease of use. Innovative extraction technologies must be further investigated to help address these issues. These methods can improve extraction efficiency and sensory properties to produce value-added protein components from nonconventional sources. Future studies should also focus on physical and biological modification methods to improve new protein functionality. The focus must be on reducing the negative impact of nonconventional proteins on final product quality, especially textural and sensory characteristics.

Further optimization is essential to enable nonconventional proteins in various food applications. Innovative processing strategies are needed to improve nonconventional protein digestibility and nutritional quality. Future research should focus on exploring and incorporating these improvements to provide sustainable and nutritional protein alternatives for food applications.

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Notes

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