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REVIEW ARTICLE

Plant-based meat analogues- green technologies in protein extraction and production technologies of plant-based meat analogues

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Abstract

Plant derived meat products are mimicked products that imitate animal meat analogue characteristics with nutritional qualities, sensory characteristics and health benefits. There is an increased demand for plant-derived meat analogue and meat alternatives, including the health, nutritional environmental or ethical aspects. This review aims to highlight the need for the development of plant-based meat analogues as future sustainable solutions to treat protein-energy malnutrition, especially among the children and vegetarians. Present trends in protein-rich plant sources, novel protein extraction methods, production technologies of plant-based meat analogues, consumer acceptability and challenges in development of plant-based-meat analogues are discussed. A single protein extraction method or with a combination with other extraction methods may results in the increased protein content and yield. When comparing enzyme assistance extraction with conventional methods, it gives highest protein content, better physicochemical properties and protein solubility improved. The high intensity ultrasound effects result in improved foaming ability of Pea protein isolation by reducing the surface tension at the air-water interface and have the potential to be implemented to modify foaming properties. Protein yield and protein percentage can be increased by defatting the raw pulse flour before extracting the proteins using conventional and modern protein extraction methods, especially ultrasound-assisted protein extraction and micro-wave-assisted protein extraction methods. Therefore, protein extraction depends on plant sources, extraction methods and processing technologies, which influence the functional characteristics of the end product.

Keywords: extraction; meat alternatives; meat analogue; microwave; plant meat; protein; ultrasound

Introduction

Meat analogy is defined as using non-meat products as the primary element. It is also known as imitation, mimic, or fake of meat products. The present trend was greater emphasis on meat analogues because consumers increased awareness of the relevance of what they eat for their health and the environment. This change was brought about by flexitarians, diseases to animals, the destruction of natural resources, low-calorie and low-fat diets and the need to reduce global warming (1). One strategy to address the populations' protein needs and avoid protein-energy malnutrition is to develop plant-based, high protein non- meat products, as many developing countries consider animal protein to be a limited resource. Due to ethical concerns, campaigns by animal rights and welfare organizations were to reduce the meat consumption and the increased emission of harmful gases (GHG) from the production process of animal-based foods, many consumers are now more aware of adopting the plant-based protein rich diets (2).

In addition, the worlds' population is expecting to increase to around nine billion people by 2050. Although the most of this population growth is anticipated to take place in developing nations, changes in demographics, including a notable rise in the proportion of older people, are also anticipated in high-income nations. The idea that elderly adults need more protein is becoming more widely accepted. The worlds' protein needs are expected to rise as the population gets older and greater (3). There is increasing evidence that plant-based proteins can be a beneficial dietary strategy for older adults, helping to reduce health risks associated with animal products while supporting adequate protein intake., Plant-based proteins offer rich in fiber and essential micronutrients potential health benefits. Although they are generally less anabolic than animal-derived proteins due to

lower digestibility and deficiencies in certain essential amino acids, food processing and nutritional strategies advancements have improved their quality. Techniques such as protein blending, fermentation and germination enhance the nutritional value of plant-based foods, making them particularly useful for preventing conditions like sarcopenia, diabetes, obesity and cardiovascular diseases in elderly people (4). By incorporating various plant-based protein sources, older adults can effectively meet their protein needs while benefiting from their fiber and antioxidant content. However, careful dietary planning is essential to ensure a sufficient intake of all essential amino acids, given the potential lower digestibility than some animal proteins.

Plant-based meat analogues are usually formulated with proteins derived from plants as the predominant raw material, alongside other constituents such as fat, water, polysaccharides, flavours, colours and additional additives (5). Selecting an appropriate plant protein source is key to producing a high-quality final product. However, using plant-based proteins presents several challenges, including poor water solubility, high structural complexity and sensitivity to pH, ionic strength and temperature. Additionally, plant protein allergenic components and undesirable flavors require improvement (6). These proteins often exhibit flavors described as "grassy," "green," "beany," "fatty," or "bitter," which can negatively impact their sensory characteristics and consumer acceptance (7) encountered with plant protein ingredients in case of product consistent and desirable functional characteristics, including off-flavours, low solubility, variability and purity (8). This is attributed to their capacity in distinctive structure formation, texturization, foaming ability, emulsifying capacity, fluid retention and nutritional characteristics (9, 10).

Protein sources from plants

Proteins from plants are trending in the development of different meat analogues like seitan from cereals, meat analogues from legumes, pulses and micro algae (11). Plant proteins play an vital role in structuring, binding, flavouring and colouring agents in the development of plant based meat analogues outstanding to their tech-functional characteristics (solubility, foaming, emulsification, gelling, viscosity, flavouring, binding and film formation) (12). The functionality, compositional and nutritional features of the plant proteins differ based on the source, variety and pre-processing of plant proteins(13). Plant proteins could be derived from one source or a combination of other protein sources to achieve a better techno-functionality and nutritional quality (14). The major plant-based protein sources in the development of meat analogues are oat protein (15) soy protein (16) wheat (17) faba beans(18) rapeseed protein (19) green peas(20) hemp seed, Sunflower meal/cake (11), pea (21) Quinoa (22) chickpea(23). The techno-functionality of the protein can exhibit considerable diversity depends on processing condition utilized during extraction and the standardization of protein and its technological characteristics (24). Expanding these array of raw materials suitable for producing meat alternatives while preserving the premium characteristics of final product involves considering various coloring agents (e.g., red cabbage leghaemoglobin, red beets, , etc.) and flavorings agents (e.g., spices & herbs) to duplicate the profile of colour and also flavour similar to meat and to hide undesirable flavours from certain legume proteins. Attributes like tenderness, juiciness and sensory qualities of meat-based products are achieved through the incorporation of fats/oils (25).

Methods of extraction of protein

Protein is an principle component of all bodily organs and tissues which plays a significant role in maintaining human health. Now a days, plant-based protein sources are gaining more importance more than animal proteins with higher ethical concerns, the growing responsibility of animal welfare organizations regarding meat protein sources and the increased burden of greenhouse emissions from animal-based proteins. The demand for plant protein-based products is high and is expected to grow significantly over the next decade. Several factors are driving this increasing popularity, including: (i) the potential health benefits of a plant-based diet; (ii) concerns about the negative health effects of high animal protein consumption, such as increased saturated fat intake; (iii) growing consumer awareness of the need for more environmentally sustainable food production; (iv) ethical considerations related to animal welfare; and (v) the general perception of protein as a beneficial nutrient, with a "more is better" mindset (26).

Some of the characteristics of the plant proteins considered as crucial for food application matrices for their approximate components, amino acid profile, shape, sequence, size, net charge, isoelectric point, solubility, heat stability, foaming, emulsification, hydrophobicity and water/oil absorption capacity. These characteristics are generally affected by the method of isolation and precipitation (6). Currently, many protein extraction methods such as water based extraction, salt-based extractions, acid based extraction, ultra filtration/diafiltration, alkaline extraction followed by isoelectric precipitation and dry fractionation technologies was used individually and/or in combination for extracting proteins. But, these techniques have some limitations, including changes in protein techno-functional properties, poor extraction yields, low stability, reduced nutritional quality, undesirable colour and generation of wastewater, which may lead to environmental hazards. So, there is a need to develop a novel extraction and eco-friendly technologies to replace the conventional methods with scientific and technological limitations. In this context, it includes enzyme-assisted extraction (27), Ultrasonic-assisted extraction (28), Reverse micelles extraction (29), Deep eutectic solvent extraction (30), Microwave-assisted extraction (31), Pulse electric field extraction (32), Sub critical water extraction and High pressure assisted extraction (33) was demonstrated trustworthy results for extracting plants proteins with enhanced techno-functionality. In addition, they are recognized as safe, affordable and eco-friendly protein extraction methods from plants. Therefore, they could provide a clean label status (34). Even though these green technologies were not completely exploited in protein extraction. This review meets the requirement to assemble the recent information about the status of novel protein extraction methods are discussed. Conventional methods (wet protein extraction and dry fractionation) were usually employed for plant protein extraction (35).

Wet Fractionation of Pulse Proteins

Wet protein extraction is generally used to extract protein-rich fractions from pulses. This procedure involves multiple phases like grinding or milling, a defatting step is usually performed using methods like solvent extraction or cold pressing to produce defatted bean flour. The bean flour is then combined with water to remove the insoluble fibres in an alkaline (pH 9) environment and the soluble fibres will be removed by protein precipitation at the isoelectric point (i.e., pH 4.5–4.8). The resultant slurry is dried to produce protein isolate contains a yield range of 60 to 90 % and a protein concentration more than 90 % (36).

Water-based extraction

The water-based extraction method is used to extract plant proteins from whole grains, either in their whole form or as dehulled grain flour. In this approach, water serves as the solvent and the starting material is soaked for a predetermined period. The pH is adjusted-preferably to neutral (pH 7) and the mixture is maintained at a specific temperature to enhance protein solubility. Following this, the mixture is homogenized, drained, or filtered to remove excess water and then processed further by churning for a set duration.

To refine the extracted proteins, a series of separation and purification techniques are employed, including affinity chromatography, membrane filtration, column chromatography, microfiltration, ultrafiltration and ultracentrifugation. The final protein concentrate or isolate is obtained through freeze-drying or spray-drying.

Research indicates that protein yield significantly increased when water-based extraction was combined with a hybrid sonication/thermal treatment approach compared to sonication alone (37). This enhanced method resulted in a higher protein concentration while preserving the proteins' structural integrity and overall quality. Water-based extraction method with the aid of hybrid sonication/thermal treatment approach, provided a higher protein concentration without significantly affecting the protein structure or quality.

Alkaline extraction followed by isoelectric precipitation

Because of its simplicity and affordability, alkaline extraction followed by the isoelectric precipitation technique is widely used for obtaining proteins from plants.

Plant-based proteins can be derived from a wide range of sources, including legumes (peas, lentils, beans, soybeans and lupins), cereals (corn, barley, rice, wheat and oats), green leaves (alfalfa, tea, radish and duckweed), oilseeds (sunflower, cotton, flax, canola, hemp and sesame), nuts (almonds, walnuts, pistachios, cashews and peanuts) and pseudo-cereals (amaranth, quinoa and chia) (37).

Additionally, proteins suitable for plant-based food applications can be obtained from algae, microalgae and microbial fermentation. They can also be extracted from food industry byproducts and waste materials, contributing to a more sustainable food supply chain (26). Low-cost food sources often utilize alkaline solubilization and isoelectric precipitation for protein recovery, as this method provides the highest yield and results in lipid-free, functional and stable proteins. The solubility of proteins in an alkaline medium is essential for the isoelectric precipitation process, where proteins precipitate at an isoelectric point of pH 4.5. This method primarily isolates globulin, as albumin and globulin have distinct isoelectric points.

Research indicates that nitrogen (N) extraction yield from biomass using alkaline extraction ranged from 5.32 % to 52.96 % (mean: 16.03 %), significantly higher than the control extraction, which ranged from 1.59 % to 25.22 % (mean: 6.61 %) (38). The alkaline extraction method increased N yield and improved the concentration and solubility of protein extracts across different biomass batches compared to extraction at neutral pH. Protein recovery efficiency varied depending on the extraction method. Alkaline solvent extraction provided superior protein recovery (60-78 %) compared to NaCl solution (20-48 %) and control extractions (21 %). The concentration of alkali or salt (0.25-1 mol L^{-1}) had a minor but significant impact on yield. While alkali solvents resulted in higher efficiency, water-based extraction produced protein extracts with the highest proportion of essential amino acids (39).

Acid extraction

The procedures for alkaline and acid extraction are comparable. Acidic extraction uses acidic solutions, such as acetone, butanol, pentanol and hexane. Acid causes a positive charge to be produced, then the pH of the protein solution to progressively drop below the isoelectric point and protein solubility will be raised. Next, soluble proteins are combined and purified by centrifugation, filtration, or precipitation once pH is brought to the proteins' isoelectric point (40). Reagents used for salt extraction are often neutral pH salt solutions, like sodium potassium or calcium chloride**.** During conventional protein extraction methods, protein loss can vary significantly depending on the specific technique and sample type. Still, a typical range might be between 20 % and 50 % of the total protein present in the original sample, with some methods potentially leading to even higher losses, especially when dealing with sensitive proteins or complex tissue matrices (6). The basic idea behind salt extraction is that proteins precipitate due to the processes of salinization and salting-out. Then insoluble debris is eliminated by centrifugation, sedimentation, decantation and screening. Next, the supernatant is desalted and dried to extract protein. Because they become soluble and contain fewer denatured as well as aggregated proteins, salt-extracted proteins are recommended for uses like foaming, gelling and emulsifying properties because they are more soluble and contain less denatured and aggregated proteins (41). This method is less effective for extracting certain plant proteins, particularly those rich in hydrophobic amino acid residues like leucine and isoleucine, due to their low solubility in water (42). However, the energy consumption, environmental contamination and hazardous solvent residues associated with classic wet extraction procedures pose limitations. Moreover, poor extraction quality and low protein extraction rates can result from using conventional procedures. Alkaline extraction alters the structures of amino acids, decreases the protein digestibility and adds a bitter taste. Acid extraction is not ideal, as it negatively impacts the solubility and gel-forming properties of the extracted proteins (6).

Dry fractionation method

Mechanically separating the proteins from other components according to their particle size and density, a process known as dry fractionation method is used to create protein concentrates. This technique separates proteins from carbohydrates and other components by first finely milling pulses. The air classification process separates the milled flour into two different fractions: (i) coarse fraction rich in proteins and (ii) fine fraction rich in starch. The protein-rich fraction could be employed as an ingredient in the production of meat alternatives and typically has a protein concentration of 40–65 %. Pulses and legumes, such as peas, faba beans, chickpeas, mung beans and lentils, have been successfully fractionated using dry methods (43).

Compared to wet fractionation, dry fractionation method offers several benefits, especially in reserving the protein structure and its functionality. The dry fractionation method does not generate effluent and does not require chemicals and large amounts of water, unlike the wet fractionation process. As a result, it reduces water use, eliminates the need for energy-intensive drying procedures and preserves proteins' natural structure and functioning (68). This methods' disadvantage is it often yields lower protein content and yield (45).

Green technologies of protein extraction

Ultrasound-assisted protein extraction (UAE)

This unconventional and non-thermal technique for extraction of proteins subjects these plant sources to the high-intensity and low-frequency sound waves Ultrasound waves (UW) with a frequency more than 20 kHz can carry high energy while travelling through a medium (46). This method works on the principle of the 'cavitation' phenomenon that comprises a cascade of events: (1) formation of air bubbles in the liquid phase; (2) volume expansion of air bubbles and (3) explosion of air bubbles. This creates high shearing forces in extraction medium, improves solubility of its compounds to be extracted by imposing high stress and deformation of cellular structure and enhances mass transfer of compounds into the extraction medium (47). Further, the bubble collapse improves the mass transfer by the creation of microchannels. It is an eco-friendly novel technology in plant protein extraction in the field food science. It also used as a pretreatment method and combined with other methods like microwave assisted techniques, enzymatic extraction methods etc. The major aim of ultrasonic assisted protein extraction is to minimize solvent consumption, extraction time, produce homogeneous mixtures, costs which increases the rate of energy transfer, reduces temperature gradient, provides selective extraction of proteins, reduces the device size and enables faster response to process control in a better way (48). Formation of hydrogen bonds and hydrophobic interactions among the proteins are influenced by ultrasound cavitation will improve the techno-functional characteristics of the protein isolate. Unlike animal food processing, producing plant-based protein substitutes involves several key considerations. First, the optimization process must be efficient, cost-effective and environmentally sustainable. Additionally, plant proteins should be modified to exhibit properties comparable to those of animal proteins. Lastly, extraction and processing techniques must focus on preserving and enhancing the nutritional value of the final product (42).

Regarding plant-based meat, this study first explores various methods for protein extraction from plants and their processing techniques. It then summarizes the role of nutritional components in plant-based products, analyzes changes in nutritional content during processing and discusses associated challenges and future prospects. By integrating insights from nutrition, food processing and analytical chemistry, this research aims to identify key control points for understanding how nutritional components evolve throughout the processing of plant-based meat (42).

Enzyme Assistance Extraction

This is a well-founded method for sparingly recuperate fine quality plant-based proteins and increases protein yield (49). Food-grade enzyme preparations, such as carbohydrases (enzymes that break down carbohydrates into simpler forms) and proteases (enzymes that break down proteins), can enhance the extraction of proteins from plant sources. This method is carried over by hydrolytic action of enzyme on the major cell wall components, like cellulose, hemicellulose and pectins, to deliver the cellular proteins.

Application of various enzymes, notably protease, is essential for the protein extraction method as it significantly enhances protein yield while concurrently mitigating protein degradation. The function of protease is to augment protein yield by dissociating proteins from the polysaccharide membrane matrix. The disruption of cell wall is a critical step that promotes the liberation of intracellular proteins. Following this liberation, proteases catalyze the hydrolysis of high molecular weight of the proteins into smaller particles with more soluble fractions, thereby establishing the conditions conducive to efficient extraction. Moreover, proteases operate most effectively at an optimal pH level, which is crucial to prevent the proteins denaturation (50). An optimal concentration of protease, typically ranging from 1 % to 5 % grams or milli litres of enzyme per gram of substrate, is advisable for the extraction procedure. Additionally, the incorporation of enzymes serves to inhibit the complex mixture formation between the released proteins from different cellular constituents, like carbohydrates under controlled physiological conditions (51).

The integration of enzymatic assistance in extraction process, coupled with mechanical methodologies as ultrasound-assisted and microwave assisted treatment, significantly enhances protein extracts' quality and yield. Commercial protein concentrates typically comprise proteins solubilized within an aqueous medium. Consequently, water is identified as the solvent of choice for optimizing extraction yields in enzyme-assisted aqueous protein extraction (52). Pectinases, cellulases, hemicellulases, and amylases are prevalent enzymes utilized in enzymatic assistance extraction (29). A singular enzyme or combination of multiple enzymes may be used. When compared to nanoparticles derived from proteins extracted via alkaline methods, those sourced by enzymatically extracted proteins exhibit superior structural and functional characteristics.

Pectinases

Pectinases are a group of enzymes that degrade pectin, a polysaccharide found in plant cell walls. They achieve this through hydrolysis, trans-elimination and de-esterification reactions, breaking down the pectins' ester bonds between carboxyl and methyl groups. This degradation process disrupts the pectin-rich middle lamella, which acts as a cementing agent between plant cells, thereby loosening the cell wall structure. In the context of protein extraction, applying pectinase facilitates the breakdown of plant cell walls, enhancing the release of intracellular proteins. By hydrolyzing pectin, pectinase reduces the integrity and rigidity of the cell wall matrix, allowing for more efficient extraction of proteins and other intracellular compounds. This enzymatic treatment is particularly beneficial in processing plant materials where pectin is a significant cell wall component.

Scientific studies have demonstrated the efficacy of pectinase in extracting valuable compounds from plant byproducts. For instance, research has shown that cellulase and protease preparations can extract pectins from various plant byproducts, highlighting the role of these enzymes in breaking down cell wall components to release intracellular substances. This enzymatic approach enhances extraction efficiency and is widely utilized in various industrial applications involving plant material processing(53).

Cellulases

Cellulases are enzymes that catalyze the hydrolysis of cellulose, a primary structural component of plant cell walls. By breaking down cellulose into simpler sugars, cellulases facilitate the disruption of the rigid plant cell wall matrix, thereby enhancing the release of intracellular proteins during extraction processes. The cellulase enzyme system comprises three main types of enzymes, each contributing to the degradation of cellulose: Endocellulases, Exocellulases (Cellobiohydrolases) and β-Glucosidases. In protein extraction protocols, cellulases are employed to degrade the cellulose component of plant cell walls enzymatically. This degradation loosens the cell wall structure, facilitating the release of intracellular proteins and other biomolecules. Using cellulases offers a controlled and efficient method for cell wall disruption, minimizing potential damage to target proteins that might occur with mechanical disruption techniques. Research indicates that cellulase and protease preparations are used to extract pectins from various plant byproducts. The research highlighted that enzymatic treatments could achieve higher extraction yields than traditional acid extraction methods, underscoring the effectiveness of cellulases in breaking down complex polysaccharides within the plant cell wall matrix (53).

Hemicellulases

Hemicellulases are a group of enzymes that degrade hemicellulose, a complex polysaccharide present in plant cell walls. Hemicellulose consists of various sugar monomers, including xylose, mannose, galactose, rhamnose and arabinose, linked in diverse branching structures. The primary types of hemicellulases include xylanases, mannanases and arabinofuranosidases, each targeting specific components of hemicellulose. In protein extraction processes, hemicellulases facilitate the breakdown of hemicellulose, thereby loosening the plant cell wall matrix and enhancing the release of intracellular proteins. By hydrolyzing the complex carbohydrates in hemicellulose, these enzymes reduce the structural integrity of the cell wall, making it more permeable and allowing proteins to be more readily extracted. In one study explored enzyme-assisted water extraction of oil and protein from rice bran using different enzymes, including hemicellulase. The results indicated that while proteolytic enzymes like Alcalase significantly enhanced oil and protein extraction yields, hemicellulase did not substantially affect yields but increased the level of reducing sugars in the extract. This increase in reducing sugars indicates the breakdown of hemicellulose, which can aid in loosening the cell wall matrix and potentially facilitate the release of intracellular components(54).

Furthermore, a study on the extraction of dietary fiber from artichoke (*Cynara cardunculus*) wastes evaluated the action of protease and hemicellulase. The findings revealed that higher concentrations of hemicellulase led to increased yields and carbohydrate content in the extracted fractions, highlighting the enzymes' role in degrading hemicellulose and modifying the cell wall structure.

Amylases

Amylases are enzymes that catalyze the hydrolysis of starch into simpler sugars. Amylases can play a supportive role in protein extraction from plant materials by breaking down starch components, thereby facilitating the release of intracellular proteins. The use of α-amylase in extracting protein concentrates from radish (*Raphanus sativus* L.) leaves. The research demonstrated that α-amylase treatment effectively degraded starch, leading to an enhanced yield of protein concentrates. This suggests that the enzymatic breakdown of starch by amylases can improve the efficiency of protein extraction processes (55).

Proteases

Proteases augment the protein yield by unbinding the proteins from the polysaccharide matrix. Cell wall degradation aids in the release of cellular proteins. After releasing these proteins, proteases fractionate the high molecular weight proteins into smaller and more sol uble portions, thus providing favourable extraction conditions. Moreover, proteases work under optimum pH, avoiding protein denaturation. A typical 1-5 % g or mL enzyme/g substrate dose of protease is optimal for various extraction procedures. Under specific physiological conditions, these enzymes can also prevent the complex formation between the released proteins and distinct cellular components like carbohydrates and phytates (50).

Microwave-assisted extraction

Microwave-assisted protein extraction (MAE) is widely recognized for isolating proteins with rigid structures that are challenging to break down using enzymatic or ultrasonic digestion. This technique is one of the most commonly employed solid-liquid extraction methods in microwave treatment due to its efficiency, practicality and cost-effectiveness.

MAE has been applied to extract rice bran protein using response surface methodology (RSM). The optimal conditions identified were 1000 W microwave power, 90 seconds of

extraction time and a solid-to-liquid ratio of 0.89 g rice bran per 10 mL of distilled water. The protein yield from MAE was approximately 1.54 times higher than that obtained through alkaline extraction (ALK) ($P < 0.05$), while the protein digestibility remained similar (56).

Optimization of MAE has been explored using different parameters, including the solid-to-solvent ratio (1:10–1:40), pH (7–10), microwave power (30 W, 50 W, 70 W), extraction time (30 seconds–8 minutes) and moisture content or pre-leaching effects. Maximum protein recovery was achieved with 50 W microwave power, a 1:30 solid-to-solvent ratio and pH 10 after 2 minutes of microwave irradiation. MAE provided a higher protein yield in less time than conventional extraction methods. SDS-PAGE analysis confirmed that watermelon seed proteins (WSP) extracted via MAE had molecular weights ranging from 25 to 250 kDa. A comparative study demonstrated that MAE achieved 90 % protein recovery in just 2 minutes with a 1:30 (w/v) solid-to-solvent ratio. In contrast, ultrasound extraction yielded 87 % in 9 minutes with a 1:50 (w/v) ratio and batch extraction provided 72 % in 25 minutes with a 1:70 (w/v) ratio. The functional properties of watermelon seed proteins obtained through MAE were superior to those extracted using conventional methods, highlighting their potential for food applications (57).

However, MAE generates significant thermal energy, which may degrade heat-sensitive bioactive compounds and reduce their effectiveness in protein extraction. Alternative strategies such as utilizing short microwave pulses or refining microwave operational parameters can be employed to enhance plant protein extraction efficiency while minimizing heat damage.

Microwaves are conventionally defined as non-ionizing electromagnetic radiation with frequencies ranging from 300 MHz to 300 GHz. They facilitate the thermal excitation of the sample via ionic conduction and dipole rotation mechanisms, thereby disrupting the hydrogen bonds within the cellular structures of plant matrix. This type of phenomenon enhances the porosity of the cell walls and promotes solvent infiltration into the cellular compartments, thereby enabling the effective liberation of intracellular plant constituents into the solvent medium. The principle advantage of the microwave extraction technique is its brevity in extraction time along with minimal solvent utilization. The sustained application of microwaves and various physical or biochemical methodologies can potentially augment the process efficiency of protein extraction.

There are two major types of microwaves; closed and open vessels. Closed vessel systems rely on controlled temperature and pressure, whereas in an open vessel system, only the part of the extraction vessel containing the sample is focused for microwave irradiation (22). Recently, solvent-free microwave hydrodistillation (SFME) was adopted for laboratory -scale applications for the extraction of essential oils from different plants and fruits as an environmentally friendly and sustainable alternative (58)

Reverse micelles extraction

Encapsulated water molecules serve as the inner cores of reverse micelles (RMs), nanometer-sized aggregates of surfactants in a bulk nonpolar solvent. Protein and enzyme extraction and purification are the two main uses of this method in food science (59). This method typically involves two phases: a forward extraction and a backward extraction. Proteins are dissolved into the aqueous cores of reverse micelles through a process called forward extraction. Additionally, the solubilized proteins from reverse micelles can be recovered via backward extraction (59). The reverse micelles system is an innovative technique for creating enzyme-immobilized magnetic nanoparticles that can boost enzymatic activity and enhance their longevity. Usually, a solution of immobilized magnetic nanoparticles is mixed with the enzyme first. At a subsequent time, this mixed aqueous solution is added in the reverse micelle system with vigorously stirring. At the end, the enzyme-immobilized magnetic nanoparticles will be obtained by an external magnetic field. Micelle allows the solubilization of selected biomolecules inside the inner water core. Hence, the extraction of the targeted biomolecule can be performed efficiently.

Deep eutectic solvent protein extraction method (DES)

The deep eutectic solvent protein extraction method (DES) is a recently developed protein extraction solvent that has opened up new research avenues with its high sustainability, cheap cost, biodegradability and nontoxicity. A deep eutectic solvent is a mixture of two or more ionic and non-ionic compounds in a specific molar ratio in which the individual melting points of the compounds must be lowered to a common eutectic point by an external force, such as heating, stirring, mechanical forces, sonication, or microwave. The special chemical characteristics of deep eutectic solvents allow it to be used in metal and food processing applications. The beneficial qualities of deep eutectic solvents and the growing need for environmentally friendly procedures in the context of green and sustainable chemistry have made it possible to employ this technique as a substitute for traditional organic solvents in a variety of disciplines.

Abbott and colleagues first introduced the term "deep eutectic solvent" (DES) in 2003 (60). When choline chloride (melting point: 302°C) is combined with urea (melting point: 133°C) in a 1:2 molar ratio, a eutectic mixture is formed with a significantly lower melting point of 12°C, much lower than that of its individual components (60). DES is created by mixing a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA) in a specific molar ratio, leading to the formation of hydrogen bonds (61). Common HBDs include organic acids, polyols, amides and sugars, while HBAs typically consist of quaternary ammonium bases, amino acids and metal ions (62).

The general formula of DES is represented as $Cat^+X^-{\cdot}zY$, where Cat^+ refers to ammonium, phosphonium, or sulfonium cations and X^- is a Lewis base, usually a halide anion. Y represents a Lewis or Brønsted acid, forming a complex anionic structure with X^-, while z indicates the number of Y molecules interacting with the anion (63). The use of Deep eutectic solvent method for protein extraction from plants offers several advantages such as environmental sustainability, low processing cost and favourable solvent characteristics such as broad range of polarity, low volatility, vapour pressure and toxicity, high chemical and thermal stability, inflammability, biodegradability and so on. In addition, as their constituents

react through the intermolecular forces than covalent or ionic interactions, these DES act as an effective alternative to replace ionic liquids and other conventional/corrosive solvents (e.g. sodium hydroxide, hydrochloric acid, sulfuric acid). Thus, Deep eutectic solvent protein extraction is a promising green approach for plant protein sources.

Pulsed electric field (PEF)

It contains non-thermal properties, shorter processing time, chemical-free processing, lower energy consumption, increased yield and environmental friendliness; it has a higher average than traditional solvent extraction techniques. These factors collectively have positioned this technology as innovative for extracting plant-based proteins . Pulsed electric field extraction(PEF) represents an innovative methodology that eschews thermal application in the extraction of proteinsThe primary advantage of pulsed electric field (PEF)-assisted extraction is its ability to modify cell permeability while minimizing thermal degradation, thereby preserving heat-sensitive compounds (64).

In artificial meat production, the nature of the protein used is crucial as it directly impacts the texture, taste and overall quality of the final product, with key factors including the protein source (animal or plant-based), its amino acid profile and ability to form a desired structure when processed, allowing for the creation of meat-like characteristics like juiciness and chewiness. By increasing cell permeability, PEF enhances the mass transfer of intracellular components. As a result, this innovative technology reduces the need for high temperatures and large quantities of solvents, lowering environmental impact and improving energy efficiency. Additionally, due to the low processing temperature and short extraction time, PEF causes minimal alterations to the nutritional and sensory properties of the final product (65). This novel plant protein extraction methodology entails applying a series of intense electric field pulses, from the range 10 to 80 kV cm^{-1}, to the plant material for brief intervals, spanning from several microseconds to multiple milliseconds. Throughout this process, a vegetative matrix will be interposed between two electrodes, resulting in the generation of a voltage to transmembrane throughout the cellular membrane, which is contingent upon the strength of the electric field, the cellular radius and orientation of the membrane relative to the directional vector of the electric field . Increased protein recovery is observed during pulsed electric field protein extraction under conditions of low temperature, extended pulse durations and elevated electric field intensities. To ensure the extraction of proteins from their native state, it will be imperative to the further adjust the input variables of pulsed electric field method. When compared with conventional thermal treatments, this method emerges as a promising alternative, as it significantly preserves the integrity of the protein throughout both the processing phase and storage duration (22). This protein extraction technique offers advantages such as improved mass transfer, elevated extraction yields, reduced processing times, minimal protein degradation and lower energy expenditures (66).

High pressure-assisted protein extraction method

High-pressure processing (HPP) showed promising novel technology for food preservation and processing. High pressure-assisted extraction (HPAE) is a non-thermal approach for production of protein that subjects a feed material to hydrostatic pressures upto 1000 MPa under controlled time and temperature conditions. After mixing the starting material with the extraction media by placing it inside pressure vessel, these pressure will be increased from surroundings to a predefined level ranging between 100 to 1000 MPa within a short duration. As pressure increased, the differential pressure between the intracellular and extracellular environments will be increased, which leads to cell deformation and cell wall damage. The solvent penetrates through the damaged cell wall and membrane into the cell, increasing the mass transfer of soluble compounds (27). HPAE is effective in improving protein functionality and digestibility, besides inactivating their antinutritional factors (67). High-pressure processing (HPP) is a non-thermal technology that modifies the food matrix, improving water diffusivity and potentially facilitating starch gelatinization. In a study on common beans (*Phaseolus vulgaris* L.), HPP was applied at pressures up to 600 MPa to evaluate its impact on hydration, drying, rehydration, cooking time and texture.

The results demonstrated that HPP accelerated hydration by up to 4.7 times, increased water diffusivity during drying by 27 % and enhanced rehydration, making it up to 2.1 times faster. Additionally, it reduced cooking time by 15 minutes due to increased initial hydration. HPP has shown potential as an effective method for reducing preparation time and improving drying efficiency, particularly in proteins derived from cereals and legumes.

Subcritical water extraction method

Subcritical water extraction method is a modern advancements in protein extraction. This technology offers lower production cost, chemical-free and shorter production times than conventional methods (68). Subcritical water extraction(SCW) is defined as where water is maintained in a liquid state under temperature (100–374°C) and pressure (< 22.064 MPa). Higher temperatures reduce the dielectric constant of water and weaken its hydrogen bonding to bring the subcritical water closer to less-polar organic solvents such as ethanol and methanol. Consequently, water in the subcritical state presents unique traits, such as a shift in the structure of its hydrogen bonds and an enhanced ionic product, Kw, which is three-fold higher than water under ambient conditions. The increased concentration of ionic products accelerates the production of hydronium (H_3O^+) and hydroxide (OH^-) ions, which is why SCW can act as a base or an acid catalyst (27). Due to the nontoxicity of water and the absence of liquid waste for disposal, subcritical water extraction is a green technology for extracting plant proteins. SWE is rapid, clean and less expensive than its conventional counterparts. Relative to organic solvents, subcritical water is advantageous in terms of its temperature-tunable density, concentration of ionic product and dielectric constant, which facilitate selective extraction of polar compounds at lower temperatures and less polar components at higher temperatures (68).

Production technologies of plant-based meat analogue

Plant-based meat analogues were developed through numerous innovative technologies aimed at replicating the nutritional composition, flavour and texture of the animal-derived meat products. These methods encompass high-moisture extrusion technology, shear cell technology, freeze structuring, spinning, 3D printing, and conventional and unconventional approaches like fermentation and enzymatic modifications. The manufacturing procedure entails converting non-muscular proteins into fibrous or granular structures that closely resemble muscle tissue at a microscopic level, often necessitating extensive processing methods and formulation, which could potentially influence the safety and nutritional quality of the end product.

Extrusion

To develop the fibrous meat analogues, co-rotating twin-screw extruders which is having length-to-diameter ratio greater than 20 and the most often utilized machinery. The extruder aligns the proteins, next the die further shapes them. Plant proteins are hydrated, unfolded, aligned and texturized through an extrusion sequence, which includes mixing, hydration, shearing, homogenization, compression, deaeration, heating, shape and cooling (69). Proteins interact during realignment due to hydrogen and disulphide bond formation, which helps create fibrous structure. Extrusion could be carried out at a low moisture level (low moisture extrusion; 40 %) to create meat substitutes (69). A lengthy cooling dye is attached to the end of the twin screw extruder in high moisture extrusion. This stops the expansion and allows the orientation and development of an anisotropic fibre structure in plant-based extrudates (69).

It has been shown that proteins were denatured by the extrusion processing conditions, which increased crosslinking and contributed in the formation of fibrous structures . The barrel and screw usually consist of at least 3 sections: (i) Feeding zone with conveying elements operating at temperature equal 25 °C; (ii) a mixing zone with a set of kneading and paddle blocks operating at temperature less 100 °C and (iii) a final stage, immediately preceding the die, which functions as a melting zone with conveying and kneading/ reverse elements to increase the mechanical energy input operating at temperature greater 100 °C (70). Following these multiple stages, plant proteins mixing with other components and hydrated, after which proteins will be denatured and aggregated.

Shear cell technology

Proteins from milk were sheared in a cone-in-cone configuration with transglutaminase present, it was first demonstrated that this approach may yield fiber-like structures . These results led to adopting shear cell technique for the structuring the plant proteins and the successful production of fibrous structures for blends of pea and soy proteins was reported. Shear conditions may be accurately adjusted to produce a different structures, as a primary benefit of shear cells over extrusion technologies (71). Furthermore, the shear cell makes it possible to produce thicker meat/fish analogues that more nearly mimic whole cuts (71).

But, because its' a batch method requiring a different pre-mixing phase, the current production capacity is constrained. Two different shear cell designs have been documented. A conical cone-in-cone device creates a sealed gap where the protein mixes are sheared in the first design. This device comprises of an upper stationary cone that is lowered and a lower rotatory cone that is heated. Shear rates between 0 and 100 rpm and temperatures up to 140 °C for long time are typical processing conditions (72). A heated stationary outer cylinder with a lid and a heated inner cylinder that rotates via a driving shaft make up the second systems' cylinder-in-cylinder design (73). This study demonstrates that utilizing simple shear flow and heat in a Couette Cell is a scalable approach for creating fibrous structures in plant protein mixtures under mild conditions. A 7L Couette Cell was used to develop structured soy-based meat alternatives, with optimal processing conditions identified at 120°C, 30 ± 5 minutes and 25 ± 5 RPM.

Fibrous structures were confirmed through visual inspection and SEM imaging, with anisotropy indices reaching up to 3.6. Due to its reduced thickness, a smaller lab-scale version (0.14 L) yielded comparable results at lower temperatures and shorter processing times. The method is scalable, enables continuous operation and is capable of producing thick meat substitutes that resemble whole muscle cuts, such as chicken breast or beef. Energy input ranged from 8.6 to 63.1 kJ/kg, with no structural differences observed across varying product thicknesses. This technology presents no significant barriers to further upscaling, opening new opportunities for plant-based meat production (73).

Wet spinning

Wet spinning is an advanced fiber-forming technique that has gained significant attention in developing plant-based meat analogues due to its ability to create fibrous structures that mimic muscle tissue. This method involves extruding a plant protein solution through a spinneret into a coagulation bath, solidifying into continuous fibres resembling animal-derived muscle fibres' texture. The procedure of wet spinning has a rich historical background in manufacturing protein-based fibrous materials. This specific technique pushes an alkaline protein solution through spinnerets and dipped in an acidic coagulation bath to trigger the precipitation process and solidification. The resultant filaments, with an approximate thickness of 20µm, can be combined and stretched to align the structure of fibers. A pea protein-based fiber system using wet spinning, achieving improved tensile strength and elasticity, is essential for mimicking real meats' texture (71).

Electrospinning

Electrospinning has recently gained significant popularity and economic feasibility as a technology for producing extremely fine fibrils. While the electrospinning process, a polymer solution subjected to strong electric field through a hollow needle/spinneret. When the electrical force exceeds the surface tension of a solution, the electrically charged polymer solution will move towards an electrically grounded collector. During this movement, a solvents' rapid evaporation occurs, leading to the jets' elongation and deformation into extremely thin dry fibers measuring approximately 100 nm. Critical factors in electrospinning process include polymer characteristics (type, structure, molecular weight and concentration), solvent properties (surface tension, viscosity and electrical conductivity), environmental conditions (relative humidity and

temperature) etc. The effectiveness of electrospinning could be increased by blending plant proteins with spinnable polymers (74). Food-grade electrospinning is primarily utilized to produce nanofibers that serve as carriers or delivery systems for bioactive compounds, such as polyphenols and probiotics. While this technique has been widely applied to animal-derived proteins like whey, collagen, egg and gelatin, its use with plant proteins remains limited. For successful electrospinning, the polymers must exhibit high solubility, the ability to entangle effectively and a concentration sufficient to ensure adequate polymer overlap [Nieuwland]. Electrospinning of proteins presents challenges, as it requires proteins to adopt a random coil conformation rather than a globular structure. Proteins in their globular state may not sufficiently overlap, leading to weak interactions and inadequate fiber formation.

Higher protein concentrations can be used to address this limitation while maintaining solubility constraints. However, most plant-based proteins do not naturally meet the requirements for electrospinning. In their native state, they tend to be globular, while denatured plant proteins often aggregate into insoluble structures, further complicating fiber formation.

Freezing structuring

Freezing structuring involves the homogeneous mixing of proteins and other components to form a uniform emulsion. Subsequently, these mixed solutions will be shaped, frozen (to create ice crystal layers) and dried (steaming, baking, or frying). This method aids in forming a fibrous structure influenced by a botanical source of protein and its properties (e.g., water retention, solubility, gelling etc) and freezing/drying parameters (temperature and time). Textural properties of proteins may be altered by adjusting freezing parameters like freezing rate, pH levels, solid content, surface characteristics, heat transfer, confinement degree and pressure conditions (75).

Research indicates the formation of multilayered structures by combining soy protein isolates. The study found that incorporating 10 % soybean flour into the formulation resulted in dense fibre layers stacked with a porous and anisotropic structure, exhibiting tensile strength comparable to meat (76). Additionally, Reseach demonstrated the freeze structuring to develop plant-based nuggets. Their research involved texturizing a protein mixture of pea and wheat protein in a 3:1 ratio (77).

Food hydrocolloids

Innovative techniques to produce meat substitutes involve blending plant proteins with hydrocolloids. This procedure includes mixing water, vegetable-based fats or oils and hydrocolloids (e.g., methylcellulose and sodium alginate) to form a stable emulsion and colloidal solution with divalent metal cations. Fiber formation is initiated by introducing casein, which can coagulate with cations, into the emulsion to trap anisotropic structures. The resulting fibrous structures would be adjusted by varying hydrocolloid and divalent metal cation concentrations necessary for precipitation, along with micellar casein content (8).

The research examined the impact of various hydrocolloids-including high acyl gellan gum, low acyl gellan gum, high methoxyl pectin, low methoxyl pectin and xanthan gum-alongside salts such as calcium chloride ($CaCl_2$) and sodium chloride (NaCl) on soy protein isolate-based meat analogues. Their findings indicated that these hydrocolloids and salts enhanced cross-linking and structural compactness at a microscopic level, thereby improving the fibrous texture of the products. This suggests that the strategic use of hydrocolloids and salts can enhance the meat-like texture of soy-based alternatives, presenting valuable applications for the food industry.

Similarly, a scallop analogue uses pea protein and high methoxy citrus pectin as the primary protein and polysaccharide components. Heat-denatured pea protein (10 %, w/w) was blended with pectin (0-1 %, w/w) to create phase-separated biopolymer mixtures. These blends were then subjected to mild shearing at 350 rpm to generate fibrous structures, which were moulded and subsequently set by gelling the pea proteins with transglutaminase (2 %, w/w) (78).

3D printing technology

This technology has recently been employed to develop plant protein meat analogues. The fundamental concept of the technology involves extruding a mixture of plant proteins with other additives (e.g., fat, polysaccharides and spices) through a narrow nozzle to fabricate multilayered blocks (79). The resulting meat analogue undergoes a bioreactors' maturation process under certain conditions to maintain its structural integrity (79). This methodology enables the production of plant-based meat analogues that simulate the texture of muscle fibres while also allowing for the customization of nutritional content.

A study on the fortification of 3D-printed vegan meat analogues with three different mushroom varieties: reishi (*Ganoderma lucidum*), saffron milk-cap (*Lactarius deliciosus*) and oyster (*Pleurotus ostreatus*). The research evaluated the printability of these formulations and analyzed their rheological properties, microstructure, texture and sensory characteristics. The results demonstrated that incorporating these mushrooms enhanced the meat analogues' nutritional profile and umami flavor and improved their printability and structural integrity (80).

In another study, researchers investigated the development of 3D-printed meat analogues using plant-based proteins, including soy protein isolate, wheat gluten and rice protein. The study focused on optimizing the composition of these protein-based pastes to enhance their printing performance. Findings revealed that adding rice protein significantly improved the rheological properties and printability of the pastes, leading to meat analogues with superior structural stability and texture (81-88).

Life Cycle Assessment (LCA) of Plant-based foods.

Manufacturers of plant-based foods may choose to enrich their products with an essential amino acids which are deficient, or they could utilize protein blends, such as combinations of legume and cereal proteins, to ensure the balanced amino acid profile (117). Meat analogues developed by plant proteins are observed to result in reduced postprandial muscle protein

synthesis responses if compared to the consumption of an equivalent amount of plant protein (89).

Several Life Cycle Assessment (LCA) studies was carried out on Plant-derived meat analogues to pinpoint critical stages in production and evaluate their impact on the environment compared to animal-derived products. Factors such as land utilization, climate impact, water consumption and energy usage were considered. The research analyzed 43 studies, stating that meat analogues produce higher sustainability levels than animal-derived products (90). Research indicates that plant-based meat alternatives (PBMAs) can potentially lessen the environmental impact of food consumption. This can be achieved by optimizing ingredient processing, which significantly influences ecological sustainability and by efficiently utilizing resources for protein production, such as cultivating high-yield legumes.

An extensive Life Cycle Assessment (LCA) study conducted on three facilities which produce 57 different meat analogs revealed decreased the emission of Greenhouse Gases (GHG), primarily linked to production processes, followed by the farm cultivation of food components and their distribution. Meat analogues are considered a promising low-carbon alternative to conventional meat, yet data on their greenhouse gas (GHG) emissions remain limited. To address this, a partial life cycle assessment (LCA) was conducted using Simapro 8.1, covering the stages from farm to factory gate. The study estimated GHG emissions for various meat analogue products manufactured in three different factories, with an average footprint of 2.19 kg CO_2e per kg, ranging from 1.33 to 2.79 kg CO_2e per kg. The largest share of emissions (45 %) originated from manufacturing, followed by agricultural production and ingredient transportation. Despite variations in production scale, factory size and location, meat analogues demonstrated relatively low GHG emissions (91).

The manufacturing phase hold to be 80 % of the environmental footprint because of the utilization of fossil fuel-based electricity. Various studies have assessed the water footprint of meat alternatives and their effect on acidification as well as eutrophication.

Challenges in the Advancement of Plant-derived Meat analogues

After being combined, the plant material is placed in the shearing zone, which is the area between the inner and outer cylinders. For example, a combination of soy proteins was treated for 30 minutes at 120°C and 20 rpm to create the fibrous structures (73). Texturally, PBMA confront issues, such as deficient fibrous structure, chewiness and juiciness. Addressing meat flavor and mitigating beany flavor in plant protein are imperative. Furthermore, achieving a distinctive red or pink meat color remains a challenge. Plant proteins exhibit a lower content of essential amino acids. Future research directions encompass (1) shaping myofibril fibrous structures through innovative processing; (2) effectively eliminating the beany flavor; (3) developing biotechnological methodologies for leghemoglobin and plant-derived pigments; (4) optimizing amino acid composition to augment the nutritional profiles. These advancements are crucial for utilizing plant proteins in developing high-quality PBMA (92).

Although renovated meat products are successfully imitated animal meat, replicating the intricate structure of whole cuts like steak, which consist of muscle tissue and connective tissue layers, adipose tissue poses a more intricate challenge. Comprehending muscle fibres' physic-chemical and functional characteristics is particularly difficult as they have complex composition. The existing nutritional variances between plant-based and animal-derived proteins make it crucial to explore effective methods to improve the nutritional quality of plant-based meat analogues while minimizing nutrient loss during food processing. Utilizing microwave-assisted heating and high-pressure processing methods will help to preserve the nutritional quality during processing. Removing or reducing anti-nutritional components could promote the progress during processing of plant based meat analogues. Main challenge in replicating meat-like flavor lies in mimicking the flavor of meat, including maillard reaction and specific amino acids and lipids that are present (93).

But not all alternatives to animal feed are sustainable-some are even overly processed. Concerns about safety and labelling are also present; customers want regulations and clear information. This disciplines' issues are related to dietetics, nutrition, sensory science and food technology.

Furthermore, in order to attain consumer approval and avoid nutritional inadequacies in individuals who opt for this kind of diet, a sufficient selection of protein source and combination of foods is crucial. The rising popularity of plant-based diets and the associated challenges and innovations in the food industry. The authors highlight that while plant-based meat and milk alternatives are well-established, there is a growing niche for egg and seafood substitutes and novel products that do not mimic traditional animal-based foods. They emphasize that not all animal food substitutes are sustainable, with some being ultra-processed, raising concerns about safety and labelling (94).

It underscores the importance of adequate selection and combination of foods to achieve consumer acceptance while preventing nutritional deficiencies for those adopting plant-based diets. Consumer acceptance of plant-based foods is influenced by factors such as taste, texture and nutritional content. Innovations in food design and technology are essential to improve the sensory attributes of plant-based products to meet consumer expectations. They also highlight the importance of clear labeling and information to help consumers make informed choices.

The need for regulatory frameworks to ensure the safety and proper labeling of plant-based food products. They point out that some plant-based substitutes are ultra-processed, which raises concerns about their health implications. Innovations in food technology and design are crucial to making plant-based products more appealing and accessible to consumers, which could also impact production costs (94).

Conclusion

The development of plant-based meat alternatives is revolutionary outbreak in food technology industry and sustainable approach to meet the nutritional requirement of the growing population. It also helps in preventing the protein energy malnutrition in children and age related and non-communicable diseases. The selection of ingredients plays a significant role in plant-based meat analogue production technologies. Improvements in organoleptic properties through different processing technologies can come at the cost of various nutritional benefits. There is a scope for clean-label approaches to develop a delicious nutritious and affordable plant-based meat alternatives for the benefit of healthy environment.

» The future aspects of plant-based meat analogues (PBMAs) is set for significant advancements through green technologies in protein extraction and production.

» Innovations such as enzyme-assisted extraction, membrane filtration, supercritical CO_2 extraction and biofermentation will enhance protein yield, functionality and digestibility while minimizing environmental impact.

» Sustainable crop sourcing, including AI-driven precision agriculture and alt ernative protein sources like algae and duckweed, will further improve resource efficiency.

» Green processing technologies, such as cold extrusion, high-moisture extrusion, 3D food printing and bioreactor-based fermentation, will enhance texture, taste and nutritional quality.

» Advances in biopolymer engineering, molecular gastronomy and smart flavor encapsulation will improve sensory attributes, while biofortification, probiotics and next-generation fats will boost nutritional value.

» A circular economy approach, including zero-waste processing, carbon-neutral production and biodegradable packaging, will promote sustainability.

» Regulatory developments, consumer education and partnerships with traditional meat producers will drive market expansion and mainstream acceptance.

As these innovations progress, PBMAs are expected to become more competitive with traditional meat in taste, texture and cost, making them a key component of a sustainable global food system.

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Authors' contributions

IJ selected the topic, collected the literature, and pooled the information on different extraction techniques and analogous meat production technologies to frame this review article's content.GS corrected the review article and guided the writing. SK provided technical support and content to be included in the writing of the review article. PG helped collect the literature and frame the review article. PP provided monitoring and guidance in writing the review article. PM suggested and guided the selection of literature from different sources. All authors read and approved the final manuscript.

Compliance with ethical standards

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