Food Reviews International

ISSN: 8755-9129 (Print) 1525-6103 (Online) Journal homepage: www.tandfonline.com/journals/lfri20

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To cite this article: Mohammad Affan Baig, Feyisola Fisayo Ajayi, Marwa Hamdi, Waqas Baba, Fatema Hossain Brishti, Nauman Khalid, Weibaio Zhou & Sajid Maqsood (2025) Recent Research Advances in Meat Analogues: A Comprehensive Review on Production, Protein Sources, Quality Attributes, Analytical Techniques Used, and Consumer Perception, Food Reviews International, 41:1, 236-267, DOI: 10.1080/87559129.2024.2396855

To link to this article: https://doi.org/10.1080/87559129.2024.2396855

Published online: 08 Oct 2024.

FOOD REVIEWS INTERNATIONAL
2025, VOL. 41, NO. 1, 236–267
https://doi.org/10.1080/87559129.2024.2396855

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Recent Research Advances in Meat Analogues: A Comprehensive Review on Production, Protein Sources, Quality Attributes, Analytical Techniques Used, and Consumer Perception

Mohammad Affan Baig[a], Feyisola Fisayo Ajayi[a], Marwa Hamdi[a], Waqas Baba[a], Fatema Hossain Brishti[b], Nauman Khalid[c,d], Weibaio Zhou[e], and Sajid Maqsood[a]

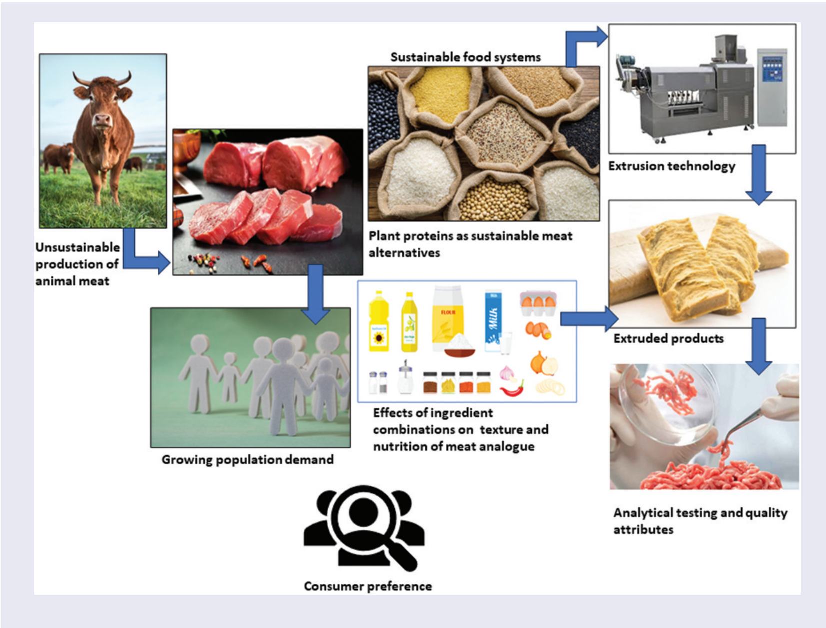
[a]Department of Food Science, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al-Ain, UAE; [b]Department of Food Technology, Faculty of Food Science & Technology, Universiti Putra Malaysia, Serdang Selangor, Malaysia; [c]Department of Food Science and Technology, School of Food and Agricultural Sciences, University of Management and Technology, Lahore, Pakistan; [d]College of Health Sciences, Abu Dhabi University, Abu Dhabi, United Arab Emirates; [e]Department of Food Science and Technology, National University of Singapore, Singapore

ABSTRACT

Recently, there has been significant research on plant-based meat as a substitute for animal meat. However, developing meat analogues with suitable techno-functional properties and a meat-like texture presents several challenges. In lieu of animal meat, plant-based meats offer a sustainable and healthy alternative. The nutritional and textural properties of meat analogues are mostly influenced by the specific type of proteins utilized and the various processing techniques implemented. Despite this, alternative proteins sourced from various plants such as legumes, pulses, and cereals, continue to be underutilized, with limited research done on their use in meat analogue development. The sustainability of production technologies and the selection of proteins can have a direct impact on both cost effectiveness and consumer of meat analogues. The challenges associated with the development of gluten-free meat analogues are currently being examined. Moreover, the extraction techniques employed in the isolation of plant proteins are of paramount importance, as they contribute to the organoleptic and textural attributes of meat analogues. By utilizing advanced technologies to sustainably utilize diverse plant proteins, it is feasible to conquer the challenges encountered in the production of plant-based meat products that are palatable to both consumers and the food industry. In this review we have discussed the functional roles of various ingredients and additives for enhancing nutritional and textural properties of meat analogues and current technologies used for texturizing plant-based meat analogues. Further we have discussed various analytical methods for testing the quality attributes of meat analogues and technological and consumer perception/acceptability-related challenges and opportunities.

KEYWORDS
Meat analogue; plant-based meats; texturizing technology; consumer perception; food sustainability

CONTACT Sajid Maqsood sajid.m@uaeu.ac.ae Department of Food Science, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al-Ain 15551, UAE



Introduction

Meat is a rich source of high-quality protein, although its consumption is associated with sustainability and public health issues, animal welfare concerns, economic limitations, and social and cultural taboos.[1–3] The problem is made even more difficult by the world's expanding population, which is projected to reach an astounding 9 million people by 2050. Recently, the scientific community has been focusing on processing plant proteins into meat-like fibrous products commonly known as meat analogues, which are considered a promising and sustainable alternative to meat. Plant-based products are associated with health and environmental benefits, fewer zoonotic outbreaks, and no animal suffering, thus attracting increasing attention from various stakeholders, including consumers, companies, researchers, and governments.[4] This is reflected by the predicted growth of the value of the plant-based meat industry from US$4.6 billion in 2018 to US$21.23 billion by 2025[5] and US $85 billion by 2030.[6] Furthermore, the demand for beef and dairy products in the United States is forecast to decrease by 80–90% by 2035 owing to the anticipated cheaper cost of plant-based foods compared with animal-derived foods.[7] Thus, a transition in protein sources from meat-to-meat analogues appears inevitable and represents a logical next step toward establishing a sustainable and secure food system.[8] Nonetheless, the production of meat analogues is a very complex affair involving restructuring of the plant proteins into a fibrous structure and texture that mimic meat. In addition, taste and appearance are also critically important for consumer acceptance of meat analogues. To simulate the techno functional characteristics of meat analogues, various processing techniques have been studied in detail. These techniques have been classified into bottom-up and top-down methods and discussed thoroughly in a classic review.[9] The fabrication of structural components into meat analogs is referred to as the bottom-up approach, while the top-down strategy uses energy input to restructure biopolymer mixtures. The authors also highlighted various commercially viable approaches for the production of meat analogues of the highest quality, such as extrusion, the use of mycoproteins, and the mixing of proteins and hydrocolloids. Some of the current re-structuring techniques utilized for texturing plant proteins are elaborated in Fig. 1.

Meat analogues can be characterized using numerous analytical techniques based on imaging, optical, and texture studies. Some of these analytical tools, such as scanning electron microscopy,

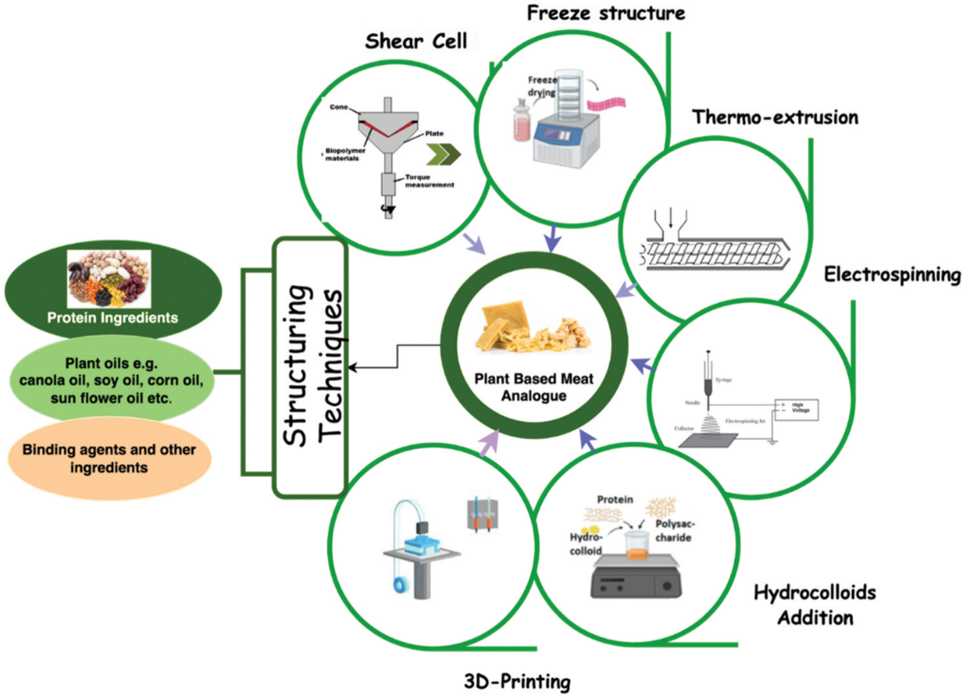


Figure 1. Current technologies used for texturizing plant-based meat analogues.

confocal laser scanning microscopy, and X-ray tomography, as well as optical techniques such as fluorescence polarization spectroscopy, image processing techniques, and the photon migration method, were recently discussed in detail.[10] Although technological, economic, and societal trends seem to be favoring a transition in protein sources from meat to plant-based meat analogues,[4] these products are yet to achieve the required consumer acceptability.[11] An in-depth assessment of plant-based meats has revealed some serious challenges that are hindering this transition, such as a lack of organoleptic and nutritional properties as well as insufficient price and industrial viability.[12] As such, it is vital to determine the influence of various ingredients and components on the organoleptic and nutritional aspects of meat analogues as well as highlight the existing lacunas in production and characterization techniques. This review article contributes novel and significant insights regarding recent progressions in meat analogues, encompassing their production techniques, factors influencing the acceptance and preference towards meat analogues among customers. Recommendations for future investigations and strategies for improving consumer perception and demand for meat analogues, were also discussed.

Background

Animal proteins and challenges due to their unsustainable production

Animal proteins, in addition to their wonderful taste, are regarded as high-quality proteins. Each year, the global demand for 325 million metric tons of meat is met by slaughtering 80 billion animals, and this demand is expected to reach 366 million metric tons by 2029.[13] The intensive animal farming needed to fulfill this enormous demand for meat and meat-related products has resulted in severe environmental consequences,[14] a loss of biodiversity, and public welfare concerns.[15] The animal meat production contributes to six times higher water footprint and elevated greenhouse gas emissions than it is for legumes.[16] Furthermore, various life-threatening diseases, such as diabetes,

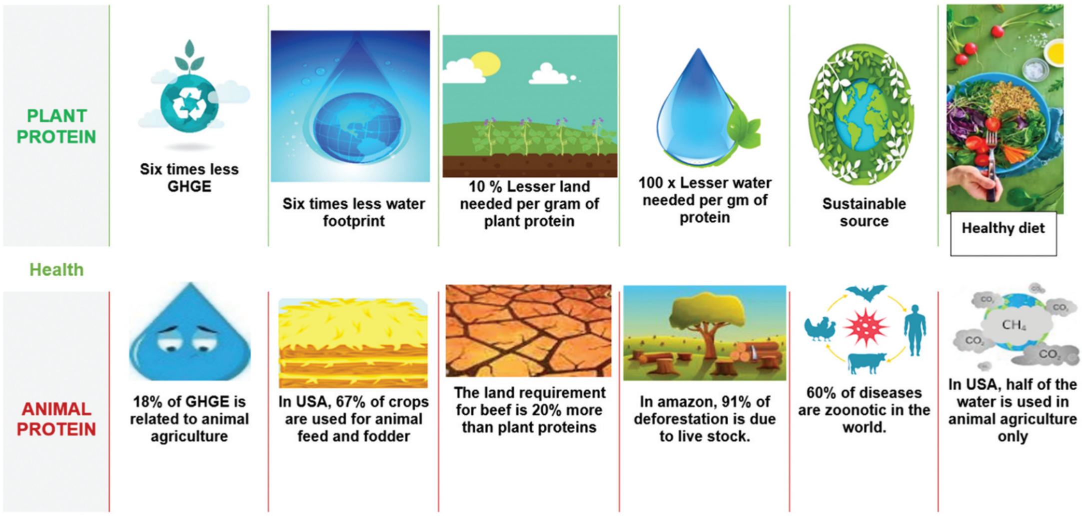


Figure 2. A comparison of sustainability between animal and plant proteins.

cardiovascular diseases[17,18] and some types of cancer,[19,20] have been linked to excessive meat intake. Newly emerging zoonotic outbreaks such as Zika virus and COVID-19 have also been associated with meat consumption.[21] The unsustainable, resource-intensive, and environmentally unfriendly nature of the meat industry has prompted many consumers worldwide to minimize consumption of meat and meat products and shift to more sustainable alternative protein sources.[22–24]

Plant proteins as a sustainable source of meat alternatives

The detrimental effects associated with the meat industry could be avoided by switching from animal to new alternative protein sources.[25] These mainly include vegetal proteins (e.g., soybean), grains (e.g., wheat, rice, millet), seeds (e.g., chia, hemp), nuts (e.g., walnuts, almonds), pulses (e.g., peas, beans, lentils), insects, and microalgae.[26] Evidently, plant proteins account for the majority of alternative proteins that could be used to replace animal proteins in the human diet. Although plant proteins do not have the same nutritional value as animal proteins, this can be overcome by blending a variety of alternative proteins.[27] Plant proteins could also ensure long-term food security and sustainability owing to their lower resource requirements; plant proteins typically require 100 times less water and 90% less land compared with the equivalent amount of edible animal proteins.[28] In addition to being more economically viable and eco-friendly, plant-based foods have numerous health advantages, including anti-obesity, anti-diabetic, and cardioprotective qualities.[29–31] Despite the potential benefits of plant-based foods compared with animal-based foods, only a very small fraction of the global population opts for a vegetarian diet. Scientists have identified various factors contributing to this, such as a lack of cooking methodologies,[8] a perceived inferior taste of plant-based meat, and technological barriers leading to environmental footprints that limit the transition from meat-to-meat alternatives.[32] This suggests that enhancing the organoleptic properties and economic viability of protein-based meat analogues may serve as the silver bullet in circumventing the multitude of concerns associated with the meat industry. Figure 2 compares the sustainability of animal and plant proteins.

Plant-based protein sources for the development of meat analogue and insights into some potential novel plant-based proteins

The most important factor governing the brand identity and distinction of a meat analogue product is undoubtedly the protein source used in its production. Proteins are essential for the physicochemical characteristics of meat analogues.[33,34] Additionally, protein digestion results in physical, chemical, and nutritional changes that depend on the protein source.[5,35] The utilization of protein constituents for the production of meat analogues is unquestionably a pivotal element for establishing the distinctiveness and distinguishing features of the product. Proteins play a crucial role in establishing structure-function correlations, specifically in relation to hydration and solubility, interfacial properties such as emulsification and foaming, flavor adherence, viscosity, gelation, texturization, as well as dough formation. Currently, the production of meat analogues uses a number of plant-based protein sources. Table 1 summarizes proteins from various plant sources and their potential characteristics for use in the manufacturing of meat analogues. The remainder of this section discusses some of the commonly used plant proteins utilized for meat analogue formation and the emerging plant proteins which have potential in manufacturing meat analogues.

Commonly used plant proteins for the formation of meat analogues

Soy protein

To date, meat analogues products have most frequently used soy protein.[56,57] The health benefits of soy protein have been thoroughly evaluated in a number of studies.[58] When compared to

Table 1. Proteins from different plant sources and their potential characteristics for use in the manufacturing of meat substitutes.

Type of protein	Protein content/ 100 g	Protein Digestibility (%)	Functional properties	Major Allergen	References
Soy proteins	~81	87.6	Hydrating capacity, solubility, colloidal stability, gelation, emulsification, foaming and adhesion/cohesion	Glycinin and β-conglycinin α-subunit	[36,37]
Pea proteins	~80	92	Water and fat binding, emulsifying, whippability, and foam stability	Vicilin and convicilin	[38,39]
Mungbean	~87.8	55	Emulsifying, foaming, water solubility, detoxifying, cholesterol lowering, anti-tumor and anti-inflammatory activities	globulin, albumin and legumin	[40,41]
Pumpkin	~82	90	Emulsifying activity, poor foaming property	2S albumin, 11S globulin	[42]
Rice	~80.6	74	High water and oil binding capacities, emulsifying properties	globulin, albumin	[43,44]
Oat	~87	86.5	Emulsifying properties, low hydration capacity, foaming property, good solubility	Avenin	[13,45]
Quinoa	~84	90	High foaming capacity, high digestibility, and low emulsifying properties	chenopodin A	[46]
Amaranthus	~82	82.4	Solubility, water and fat absorption, emulsifying capacity, and foam formation capacity	globulin, albumin	[47]
Peanut	~90	70	Emulsifying activity, emulsifying stability, foaming capacity, excellent water retention and high solubility	Ara h 1, Ara h 3, Ara h 2, Ara h 6	[48,49]
Flax	~90.6	83	Thermally stable, low water solubility, emulsifying activity, water and fat absorption	malate dehydrogenase MDH-1	[50]
Sunflower	~82	60	Emulsifying activity, low foaming and low gelling properties	2S albumin and nonspecific lipid transfer proteins (Hel a 1, Hel a 2, Hel a 3, and Hel a 6)	[51]
Sesame	~18.81	89.57	Lower protein solubility, water holding and fat binding capacities, high emulsifying and foaming properties	2S albumin	[52]
Chia	~92.5	79.8	High solubility, foaming capacity, low surface hydrophobicity	lectin, elongation factor, 11S globulin	[53]
Hemp	~86	86.7	Low emulsifying activity, low emulsion stability, low protein solubility and low water-holding capacity	Major thaumatin-like protein, Lipid transfer protein Can s 3	[54,55]

unprocessed soy protein, processed soy protein has been demonstrated to have a higher availability of key amino acids from a nutritional standpoint.[59] In fact, soy protein isolate has the highest digestibility score that can be achieved (1.00), which is equivalent to those of animal-derived foods such as meat, dairy products, and eggs.[60,61] Numerous options exist to create a variety of sophisticated products from soy proteins on account of their superior techno functional properties. In addition, soy-based foods are already widely consumed by a variety of cultures around the world.[36] Comparing foods made from minimally processed soy flour to those made from soy protein isolates, the nutritional quality of the latter is superior.[62] Soy is a versatile protein which allows for the creation of meat-like consistencies and tastes through various approaches. Such methods involve the utilization of texturizing techniques to transform soy protein into high-moisture meat analogue (HMMA) or texturized vegetable protein (TVP).[57,63]

Pea protein

Owing to its minimal allergenicity, substantial nutritional content, availability, and affordability, pea protein has received considerable attention. Peas are rich in vital vitamins and minerals, low in fat, and abundant in protein and carbohydrates.[38] The protein from peas can be employed as an innovative and versatile substitute for soy or animal proteins, which are frequently utilized in various functional food applications due to their availability, affordability, nutritional value, and health advantages. Commercial pea protein isolate (PPI), for example, is a concentrated type of pea protein with a protein content of approximately 85% on a dry weight basis.[64] PPI contains no gluten, in contrast to normal cereal proteins, making it an important gluten-free ingredient for food preparation.[65] As a novel plant protein source, pea protein provides additional ways of developing functional foods owing to its superior physicochemical properties.[66] Pea protein exhibits numerous techno-functional characteristics, including a high emulsifying capacity and stability, a moderate foaming capacity and stability, and a low gelling capacity and strength. Additionally, it possesses a high water-holding capacity, rendering it suitable for a diverse range of food applications, particularly in the realm of meat analogues. Furthermore, Pea protein has good solubility in water, a crucial attribute for the development of protein networks and emulsions.[67] Pea protein has also been used in high-moisture meat analogue products because it plays a remarkable complementary role to other protein components with specific functional features.[5,68,69] The addition of pea protein can enhance the textural and viscoelastic characteristics of meat analogues.[70]

Mung bean protein

Mung beans are rich in minerals, calcium, iron, manganese, and vitamins B and C. Mung beans tend to appeal to health-conscious people on account of their low-fat content. Despite their low calorific value, mung beans contain high protein (20–27%) with excellent digestibility. As such, consuming seeds of mung bean along with cereals has been suggested to prominently enhance the quality of consumed protein in a vegetarian diet. Mung beans are also inexpensive to grow. They could be a source of plant protein with the potential to grow into textured vegetable protein that could make up a large proportion of people's diets and be both affordable and healthy. Mung bean protein isolate has been shown to perform several useful functions in processed foods, including improving the foaming, emulsifying, and water absorption properties.[71] However, only few studies were conducted on physicochemical characteristics of mung bean protein to determine its potential in the formation of textured vegetable protein for use in meat-based products.[72] The mung bean protein isolate has high concentrations of leucine, proline, arginine, glutamic acid, and phenylalanine which make it a high-quality protein source.[71] However, according to Mubarak et al.[73] mung bean protein shares many of the same amino acids as soybean protein. Steetapan et al. used a high moisture extrusion process to make fibrous products with different ratios of mung bean protein isolate (MPI) and mung bean flour (MF).[71] They found that MF improved the extrudability and texturization of MPI, but also affected the color and texture of the products. In comparison with commonly extruded soy protein, mung bean protein exhibits several useful characteristics, including enhanced physicochemical properties such as gelling and foaming capacity and superior texturization under medium shear force due to its lower denaturation enthalpy, which prevents excessive denaturation. Overall, mung beans are an environmentally friendly plant protein source that are amenable to extrusion as a green processing technique.[42,74]

Pumpkin seed protein

Pumpkin (*Cucurbita* sp.) seeds contain a considerable amount of protein in the range of 31.5–51% and could serve as a potential source of protein for plant-based meat analogues. However, there are limited studies about the structural and functional properties of pumpkin seed protein, which restricts its use in food formulation. The variation in physicochemical characteristics that pumpkin seed proteins exhibit can greatly influence their applicability to food production on a pilot/industrial scale.[42] The techno-functional properties of pumpkin seed protein make it suitable for various food applications, especially for meat analogues. Pumpkin seed protein has a good solubility in water, high emulsifying capacity and stability, moderate foaming capacity and stability, low gelling capacity and strength and high water-holding capacity.[75,76] Ebert et al.[77] studied high moisture extrusion process to make fibrous products with different ratios of pumpkin seed protein isolate (PSPI) and pumpkin seed flour (PSF). They found that PSF improved the extrudability and texturization of PSPI, but also affected the color and texture of the products.

Rice protein

Rice has a higher protein quality than both wheat and corn, although it is slightly inferior to that of oats. The amino acid composition of rice protein was reported to be superior to those of casein and soy protein isolate.[78] The main waste product from milling is rice bran, which can be further processed to obtain oil. Protein makes up 15.4% of the defatted bran residues,[79] and the proteins extracted from heat-stabilized defatted rice bran may be used as a nutritive food ingredient.[80] Rice protein has several techno-functional properties that make it suitable for various food applications. It has good solubility in water, high emulsifying capacity and stability, moderate foaming capacity, and low gelling capacity. Rice could be a potential ingredient for meat analogue production. Manufacturing of meat analogues utilizing rice protein isolate (RPI) remains an unexplored area. RPI holds the potential to partially substitute soy protein isolate (SPI) in the production of meat analogues. The incorporation of RPI has yielded meat analogues

that possess improved nutritional properties. A novel range of meat analogues has been devised, employing RPI in conjunction with SPI through the process of extrusion cooking.[68] Qiu et al. used a mixture of soy protein, gluten, and rice protein to make pastes that could be 3D printed into meat-like shapes and textures and found that rice protein improved the printing performance, rheological behavior, and textural properties of the soy protein-gluten pastes and concluded that rice protein could be a useful ingredient for 3D printing of meat analogues.[81]

Oat proteins

Oat grains have a significantly higher protein content (15–20%) compared with other major grain categories.[82,83] In terms of nutrition, oat proteins have excellent biological value (74.5–79.6%), digestibility (90.3–94.2%), and net protein utilization rate (69.1–72.4%).[84] Additionally, oat protein has an amino acid composition that is superior to those of most cereals owing to its higher concentrations of essential amino acids lysine and threonine, which makes it more nutrient dense.[85] Oat protein is also gluten free, thus rendering it suitable for those with coeliac disease.[86,87] However, additional protein is required to create oat-based products that are a substitute for animal meat, dairy products, and bread goods to attain comparable protein content or texture.[88] Techno-functional properties of oat protein make it suitable for various food applications, especially for meat analogues. Oat protein has a good solubility in water, has a high emulsifying capacity, with moderate foaming capacity, low gelling capacity and high water-holding capacity. Kaleda et al. studied the production of meat analogs using a low-moisture extrusion process with oat and pea protein blends at varying ratios.[89] The composition of the blends was found to significantly impact various techno-functional properties. The extrusion conditions were observed to have a greater influence on hardness, fibrousness, and moisture. It was suggested that the combination of oat protein and pea protein could serve as a viable alternative to soy and gluten proteins in the manufacturing of meat analogs.

Emerging sources of proteins for utilization in meat analogue industry

Emerging plant proteins refer to those that are not frequently employed in meat analogues, but possess the capacity to provide innovative protein sources, texture, taste, and nutritional value. However, numerous challenges and prospects for additional investigation and advancement still persist, including refining the techniques of processing, enhancing the nutritional quality, diminishing the presence of anti-nutritional factors, and augmenting the consumer reception towards these novel ingredients. Protein sources such as quinoa, amaranth, peanut, flaxseed, sunflower, sesame, chia and hemp are discussed in the following section.

Quinoa is considered a superfood due to its high nutrient content. It is contemplated as one of the crops that will offer food security in the 21st century.[90] A variety of applications of quinoa proteins are made possible due to their techno functional characteristics, which include emulsifying, foaming, solubility, water-holding, and gelation properties.[91] Quinoa protein has higher emulsifying capacity and stability compared to soy, wheat and pearl millet. Quinoa protein has a low gelling capacity and high water holding capacity. Although, the use of quinoa protein in foods is limited by its indigestibility.[46] As per the variety, quinoa has a total protein content between 10.4% and 17.0%.[92] Future applications could include textured protein as an animal meat analogue and fermented foodstuffs, among many others.[91]

Amaranth protein exhibits considerable nutritional value and favorable techno-functional properties, encompassing solubility, emulsification, foaming, gelling, and water-holding capabilities. The manifestation of these attributes is subject to various factors, including the pH, ionic strength, temperature, and protein concentration within the solution, and can be influenced by enzymatic hydrolysis or chemical modification. Amaranth protein could possess the potential to form meat analogues, contingent upon the desired application. Amaranth is a non-traditional source of protein that has attracted research attention recently because of its superior nutritional value.[93]

Peanuts *(Arachis hypogaea* L.) are the fourth leading oilseed in the world. Fat-free peanut flour, which is a side product of the process used to extract oil from peanuts, contains 50–55% high-quality protein. Peanut protein has great amino acid composition, good volatility, low amounts of antinutritional components, and a reliable supply. Rehrah et al. studied the production of plant-based meat analogues using DPF using single-screw extruder with different levels of protein, moisture, temperature, and screw speed.[94] The meat analogue produced from inexpensive DPF has the potential to compete with commercial meat analogues, thereby adding value to the peanut industry. Extrusion cooking is one method for converting DPF into meat-like items by texturizing the peanut proteins into fibrous meat analogues.[94] Zhang et al. formulated the wheat gluten and peanut protein based high moisture extruded meat analogue and found that ratio of wheat gluten and peanut protein affected the color, texture, and water distribution of the meat analogues.[95]

Flaxseed is an important oilseed crop and contains a high protein content of approximately 18–22%, depending on the variety and place of origin.[96] Flax seed protein has high nutritional value and good techno-functional properties, such as solubility, emulsification, foaming, gelling, and water-holding. This makes flaxseed a desirable ingredient for the food sector. Recent research has indicated that flaxseed protein concentrate has favorable functional qualities,[97] making it a potential protein source for use as an animal meat analogue.[98]

Sunflower seeds are among the most intriguing sources of plant protein due to their high protein content, nutritional value, and functions. Comparing sunflower seeds to other plant sources, it has been found that the amount of antinutritional components in the former is relatively low. The functional qualities of sunflower seed protein are the determining factor in its applicability. It has emulsification qualities similar to soy protein isolates.[51] Sunflower seed protein, which is a byproduct of the oil industry, can be considered a good soy substitute and a potential source for preparing meat analogues.[99] It has found extensive use in the food industry because of its abundant supply, content of high-quality proteins, and functional properties, which enable its incorporation into a variety of meat analogue products. In addition, the majority of oilseed proteins are underutilized, which makes their economic valorization highly alluring. The stability of emulsions and foams containing sunflower seed proteins is comparable to that obtained with commercial soybean protein isolates.[100] Moreover, sunflower seed protein concentrates displayed comparable gelling capacity to other commercial proteins and could be utilized in various food applications.[101]

Sesame seeds and oil are widely used in the food industry. Sesame seeds have a protein content of 20–25%, and these compositional characteristics have made them one of the primary sources of edible oil and protein.[102] Sesame seed protein has high nutritional value and good techno-functional properties. Idowu et al. studied the protein fractions from sesame seeds based on their solubility.[103] They measured the functional properties of each fraction and concluded that sesame protein fractions have different characteristics and potential applications in food systems. The oil-free meal with a protein content of approximately 50% can also be used as an ingredient in functional foods and nutritional supplements owing to its numerous advantageous qualities.

Chia (*Salvia hispanica* L.) has been widely accepted by consumers around the world, and its use as whole seeds, whole flour, or even an oil is highly regarded. As a result, this oilseed is being employed as a novel ingredient in the development of new meat products owing to its good nutritional qualities and excellent techno functional properties, including its ability to hold both water and oil.[104] Chia seeds contain 25–40% oil, 17–24% protein, and 18–30% dietary fiber as key ingredients. The proteins have excellent water- and oil-retaining properties, are easily digested, and produce good foam.[105] Given that chia seeds are rich in plant-based omega-3 fatty acids, demand for them is rising in the functional food sector. Chia seeds are used for their oil, but a sizeable amount of protein is left over as a byproduct. The distinctive qualities of chia seed protein are poorly understood, despite its recently discovered nutritional relevance.

Hemp (*Cannabis sativa* L.) offers numerous advantages across a range of industries. Employing farmed hemp seeds as a source of raw materials for food manufacturing is also gaining popularity. Hemp seeds are rich in protein (20–25%) and edible oil.[57] The byproduct known as hemp seed protein or hemp seed meal is obtained after processing hemp seed into edible oil and is nutritionally equivalent to other protein sources.[106] Additionally, hemp seeds contain high protein content, and the protein concentrates have superior functional attributes such as water holding capacity, foaming capacity and gel formation. It exhibits promise as a potential substitute protein source in various food applications, particularly in the creation of meat analogues.[54,84] Zahari et al. employed a twin screw extruder in order to fabricate high-moisture meat analogues (HMMA) containing varying amounts of hemp protein concentrate (HPC) in lieu of soy protein isolate (SPI).[57]

Various factors contribute to the diverse performance of protein sources in meat analogue production, impacting the quality, sustainability, and consumer reception of the final product.[107] The following section discusses factors which affect the performance of different protein sources in the production of meat analogues.

Protein Composition: Diverse protein sources exhibit varying amino acid profiles and protein structures, impacting their functionality in meat analogues. Soy, pea, and wheat proteins are commonly utilized for their texture and nutritional value.[108]

Processing Techniques: The methodologies employed in processing plant proteins into meat analogues, such as extrusion, can affect the final product's texture, taste, and appearance. Texturized vegetable protein (TVP), frequently processed using extrusion technology, serves as a common foundation for meat analogues.[109]

Nutrient Content: The purpose often entails aligning the nutritional content of plant-based meat with that of animal meat, incorporating not only protein but also essential vitamins and minerals. Different plant proteins offer distinct nutritional advantages.[110]

Sustainability: The environmental implications of plant-based protein production can fluctuate. Variations exist in greenhouse gas emissions among different protein sources, a critical factor for producers and consumers.[111]

Technological Advances: Innovations like 3D printing of meat analogues and the creation of new protein sources from single-cell organisms or by-products are broadening the array of textures and flavors accessible in meat analogues.[112]

Consumer Preferences: The success of meat analogues heavily relies on taste, texture, and overall acceptability. Combining different protein sources can achieve the desired sensory characteristics.[113]

Functional roles of various ingredients and additives for enhancing nutritional and textural properties of meat analogues

The selection of ingredients and additives significantly influences the texture and behavior of meat analogues during processing.[114] Proteins serve as the primary structural elements in these products. Upon heating and shearing, plant proteins can unfold and create a fibrous texture reminiscent of meat. The choice of protein source (soy, pea, wheat, etc.) plays a role in determining the texture, taste, and nutritional content.[92] Binders and fillers in the form of polysaccharides are employed to aid in water retention, enhance texture, and potentially impact the viscosity of the mixture throughout processing, which is crucial for the final texture outcome.[115] Fats play a role in enhancing juiciness and flavor. The choice of fats and their melting point can influence mouthfeel and product stability when subjected to cooking temperatures.[116] Additives such as Flavors and Colorants are essential in creating an appealing product that mimics the taste and appearance of meat, requiring careful selection to withstand processing conditions.[101] Various processing techniques like extrusion or shear cell technology can modify the protein structure, leading to a meat-like fibrous texture.[117] Parameters such as temperature, pressure, and shear rate during these processes are adjusted based on ingredient properties to achieve the desired texture.[118] Processing-induced modifications, including covalent interactions, further refine the texture and stability of meat analogues.[119] The intricate relationship between ingredients and processing conditions necessitates meticulous optimization to develop a meat analogue with a pleasing texture that behaves akin to meat when cooked.[120] Ongoing research is dedicated to enhancing the quality of plant-based meat analogues by refining these mixtures and processing methods.

Easily accessible protein-rich components (primarily highly refined plant materials) may not possess the optimal functional properties for preparing meat analogues. This is reflected in the need to add supplements to enhance product texture. The protein components utilized to manufacture meat analogues are one of the most crucial factors governing the obtained techno functional and physicochemical properties.[33,34]

The preparation of meat analogues also involves the utilization of numerous lipid components (fats and oils). Vegetable and plant oils, including canola (rapeseed) oil, sunflower oil, corn oil, coconut oil, sesame oil, cocoa butter, and many others, are among the lipid components used in current meat analogues. In meat analogue formulations, these fats and oils play an important role in the juiciness, softness, mouthfeel, and flavor release.[5]

Starches and flours, which can enhance the consistency and texture profiles of meat-analogue products, are examples of ingredients that contain carbohydrates. To improve the product stability and shape, binding additives, such as methylcellulose, acacia gum, xanthan gum, carrageenan, and many others, are utilized.[13,121] These additives are intended to enhance the interaction of water with the lipid and protein parts of the processed food system. Modern meat analogues and many other kinds of processed foods contain methylcellulose, a modified form of cellulose that displays good performance as a binder in food products when added in the appropriate amount.[122]

Table 2. Functional role of different ingredients and additives for enhancing nutrition and textural properties of meat analogues.

Meat ingredient	Functional role	Examples	References
Protein	Texture, binder, base for fat substitutes, emulsifier, good gelling and water holding capacity	Soy, Pea etc.	[124]
Oils and fats	Juiciness, tenderness and flavour release	Soy, sunflower, rapeseed, canola, corn, palm, coconut and sesame oil etc.	[125]
Binding agents	Used to improve the smoothness and consistency of the product by binding water or fat	phosphates (E450), NaCl, citrus and cereal fibres etc.	[126,127]
Texturizing Agents	Use to improve the texture	methylcellulose, carrageenan, locust bean gum, calcium alginate etc.	[128]
Flavouring agents	For meat like flavour	savoury spices, meaty and savoury aromas	[129]
Colourants	Used for obtaining desired meat color	betanin and beetroot extracts, caramel colours, malt or annatto, turmin, cumin, erythrosine and carotenoids, such as carotene, canthaxanthin and lycopene	[130]
Water	hydration medium, plasticizer and reaction agent	–	[95]

Several kinds of gums, including acacia gum, xanthan gum, guar gum, and others, are employed in the production of processed meats and meat analogues. Flavors are also added to most processed and prepared foods and as ingredients in meat analogue products in the form of seasonings and spices. The method for capturing the flavor and aroma characteristics of meat products is the isolation of certain naturally occurring volatile chemicals, sometimes in conjunction with different thermal processes.[123] Table 2 summarizes the functional roles of various ingredients and additives in enhancing the nutritional and textural properties of meat analogues. Texturization and structure imitation are used to create texture and bite in plant protein-based meat analogues (PPBMAs) in an effort to imitate meat. These can help to expand the meat alternative market and promote consumer acceptance because the texture and mouthfeel of meat products are sorely sought after. Plant-based proteins must be restructured using a series of techniques to imitate the fibrillar matrix of meat muscle because plants themselves are composed of amorphous tissue. Figure 2 shows the current technologies used for texturizing plant-based meat analogues. In addition, an overview of the structure orientation, benefits, and limitations of various technologies for texturizing meat analogues is presented in Table 3.

Current technologies used for texturizing meat analogues

Texturization and structure imitation of plant protein materials creates texture and bite to plant protein-based meat analogues (PPBMAs) to imitate meat.[133] As a result, help to expand the meat alternatives market and upsurge consumer acceptance, since texture and mouthfeel like meat products are sorely sought after. Plant-based proteins must be re-structured using series of techniques to imitate the fibrillar matrix of meat muscle, as plants are made up of amorphous tissue.[107] In the following section, we discussed in detail the different techniques that are applicable for re-structuring plant protein to mimic whole muscle meats.

Extrusion technique

The use of extrusion is a regular technique for re-structuring plant-based materials into fibrous products. This thermo-mechanical process applies multiple actions viz mixing, kneading, heating, melting, and cooling, are used to produce the final texturized product. The subjection of proteins to high-temperature leads to denaturation of protein molecular structure resulting in unfolding and reorganization.[134] In practice, extrusion is cooking conducted in two ways based on the amount of water included. One approach is by incorporating low moisture of about 30% into protein concentrate/

Table 3. An overview of the pros and cons of different technologies for texturizing plant-based meat analogues.

Technique	Starting material	Structure Orientation	Advantages	Disadvantages	References
Extrusion	Protein isolates/concentrates Defatted soy flour, polysaccharides composite	Layered and fibrous matrix	High yield Economical and low-cost Versatility Enhance plant protein digestibility	Relatively energy intensive Induce color change due to Maillard browning	[9,88]
Wetspinning and electrospinning	Protein isolate and non-solvent protein solution	Fibrillar structure	Cost effective Uniform fibrils length and thickness Production can be scaled up	Difficult to control several processing parameters Requires pure protein High usage of chemical reagents Generates large amount of waste	[9,131]
Mixing of proteins and hydrocolloids	Protein isolate, hydrocolloids, cations	Fibrous matrix	Form fibrous structure that can be modulated	Require divalent metal cations and hydrocolloids for protein precipitation	[9,132]
Freeze technique	Protein isolate	Interwoven sheet-like structure	Modulate texture of plant proteins	Require several optimization and monitoring of freezing conditions	[70]
Shear flow	Protein isolates polysaccharide blends, protein isolates-calcium caseinate, protein concentrates	Layered and fibrous matrix	Inexpensive Produce unique fibrous matrix	Require more investigations for increasing scaling of production	[9,56]
In vitro culture of animal meat using tissue engineering	Skeletal muscle cells, growth medium	Muscle fiber	Produce products with similar texture to animal muscle Modification of the product nutritional content	High cost of production Require maturation under specific conditions Complex 3-D structure	[81]

isolate/flour in an extruder to obtain a final texturized product.[9] Traditional low-moisture extrusion results in a dry product with a porous and spongy-like texture with high water-binding capacity, which requires rehydration prior to further processing. Generally, with low-moisture extrusion, fibrous texture that resembles meat are impossible, thus, mostly intended to produce texturized viscous proteins.[135]

The second approach involves moistening of protein concentrates to reach moisture content of about 50–60%, which is described as wet or high-moisture extrusion cooking.[9] High-moisture extrusion (HME) can modify plant proteins to yield fibrous structured meat analogues resembling whole muscle products through three essential steps; (i) pre-conditioning of protein concentrates; (ii) transformation of conditioned proteins into viscoelastic mass (iii) immediate cooling of the end texturized product to form the anisotropic structure.[123,136] Nowadays, plant proteins such as soybeans, wheat gluten and pea are the common raw materials used as the building blocks to construct high-moisture extruded meat analogues. However, new plant ingredients like cotton seeds, peanuts, rapeseed, and sesame seeds are gaining immense attention as promising alternative ingredients.[136] Consequently, the use of extrusion cooking on these numerous ingredients could further expand the meat analogues market. In spite of its outstanding performance, the major drawback of this technology is the high usage of energy.

Wet spinning and electrospinning

Spinning technology is another alternative technique to create well-defined fibre from protein solution to mimic muscle meat. In the wet spinning process, emulsion (protein solution) is introduced into a spinneret, afterwards immersed into a coagulation bath, containing protein non-solvent, which promotes cross-links and the formation of thin fibrils of approximately 20 µm thickness.[9,123] The wet spinning operation is affected by the characteristics of the pre-spun solution, which is elaborated in more detailed studies.[137,138] As with other texturizing processes, wet spinning process is one of the standard techniques, nonetheless preferably employed for fabricating fibril. On the other hand, electrospinning process involves the application of high voltage to re-structure biopolymer solutions into nanofibers.[123] The method involves pressing viscous solution through a spinning nozzle or hollow needle under an electrical potential gradient proportional to a ground electrode. The accumulation of electrical charge consequently deform the drop (jet) of polymeric solution from the nozzle into a conical shape which extends into a thin fibre (≈100 nm).[123]

Developing spun protein is notoriously challenging, since plant proteins are mainly globular, and upon denaturation, they may not suitably unfold, resulting to insoluble aggregates and insufficient entanglements for fibre formation.[9] For electrospinning of plant proteins to occur, high temperature can be employed prior the process to unfold their globular conformation, while preventing insoluble aggregates formation. Again, combining bio-polymer solutions (proteins) with a "spinnable" polymer and using higher concentrations of protein (not beyond the maximum solubility) may be used to overcome this problem.[139] Generally, spun techniques have been utilized to produce re-structured meat analogues in combination with different food-grade reagents in combination with zein or wheat protein and polymers. Despite the vast literature information about electrospinning of proteins, only those originating from animal-based proteins are available, while information on plant proteins is sparse.[140]

Blending of proteins and hydrocolloids

Meat-like products with fibrous texture can be achieved using a patented technique of mixing plant proteins and hydrocolloids. The interactions between polymer solutions and hydrocolloids that precipitate with multivalent cations is a promising technique to texturize protein for inventing fibrous products.[141] In this process, protein(s) emulsion containing lipids and water is mixed with hydrocolloids (e.g., methylcellulose, sodium alginate, and pectin) and sheared using an extruder to form a stable emulsion. Adding hydrocolloids to the emulsion offers a better restructuring of fibre. In practice, combining various fractions of the starting materials i.e., protein and methylcellulose can achieve a very positive effect on the formation of fibre structure. Fibres are produced by adding a colloidal solution (i.e., micellar casein and divalent metal cations) to the stable emulsion, ensuring the formation of the anisotropic structures.[141,142]

The quantity of added divalent metal cations (e.g., calcium carbonate and/or calcium chloride) is preferably selected so that it is sufficient to initiate fibre formation alone or when combined with the calcium fraction of micellar casein.[143] The fibrous products are separated from the aqueous solution by pressing, thus producing about 60 wt. % dry weight. The resulting fibre structure and strength can be modified by combining specific hydrocolloids with the divalent metal cations required for precipitation.[144] For instance, it was established that increasing the concentration of hydrocolloids, particularly methylcellulose enhances the fibre yield, and results in softer fibrous products similar to fish or chicken muscle.[145] Differently, reduced concentration of methylcellulose and increased concentration of divalent metal cations or micellar casein results in firmer fibres like beef.[142] Various plant proteins including maize, soy, and rice are useful for fabricating meat substitutes using this process. In 2005, a brand name "Valess" developed meat analogous products based on casein and alginate hydrocolloids. Although this process yields final products with some degree of fibrous structure, the resources used are still very much exhaustive.[146]

Freeze structuring technique

The freezing of protein(s) slurry or aqueous solution to generate fibrous structure, is referred to as freeze structuring or freeze alignment.[109] Protein texturization by freeze technique involves freezing of protein emulsion, followed by creating perpendicular direction zones that induce ice crystals formation leading to stretched fibrous structure, and removal of moisture to yield a dry and firm fibrous protein matrix.[147] When well-mixed plant-based protein slurry is frozen by gradual heat removal, subsequent formation of ice crystals further induces an anisotropic structure.[9] The formation of aligned and highly interconnected sheet-like protein structures depends on the uniform perpendicular direction of ice crystals entrapped within the aligned protein molecules. These overlapping sheets subsequently form a cohesive fibrous product. Moreover, to obtain a similar porous and fibrous structural matrix to that of animal meat muscles, ice crystals should be subsequently removed by lyophilization. Notably, the shape and size of needle ice crystals can be directed by the freezing conditions (e.g., temperature and freezing rate).[148]

Due to plant proteins structure-functionality interactions, it is crucial to select a suitable protein because it influences the final product fibrousness during freezing structuring. It was reported that products fabricated with pea protein had more hardness as compared to wheat protein.[146] However, blending both pea and wheat protein fractions exhibited outstanding springiness. Accordingly, meat analogue with satisfactory hardness and elasticity can be produced using composites of plant-based proteins.[147] Recently, Yuliarti et al.. (2021) utilized freezing technique for the fabrication of plant-based nugget analogue, where it was found that the analogue textural profiles and sensory attributes were similar to meat exhibiting fibrous and well-layered structure.[70] For the production of unique fibrous products, excellent solubility of proteins should be retained before and throughout the process.[9] Furthermore, dehydrated end products with fibrous texture produced using freeze techniques can be rehydrated using stabilizers, fat solutions, flavors and color. The major limitation of this technique is monitoring and optimizing various freezing temperatures and rates at a given point in time.[148]

Shear flow technology

An innovative procedure based on a simple shear flow was proved efficient in plant protein structuring. The concept of this method is based on flow-induced structuring wherein plant-based polymeric mixture is subjected to a distinct shear flow at elevated temperature and high moisture.[149] In shear-induced structuring of proteins, two devices with different geometrics are used, namely cone-cone and cylindrical Couette cell setup. In cone-cone device, biopolymer is fixed in the cavity in-between the stationary top cone and rotating bottom cone, afterwards heated in an oil bath at high temperatures (120–140°C), sheared and subsequently cooled down.[150] The cylindrical Couette cell is designed so that the product is placed directly between two cylinder plates enabling shear flow. Isotropic fibrous matrix is obtained with several plant-based biopolymers combinations or blending protein with calcium caseinate or polysaccharide under varying process conditions, for example, soy protein isolate (SPI) and wheat gluten (WG), WG and pea protein, SPI-pectin, calcium caseinate etc.[151] Although, applying shear-flow on SPI alone induced the protein structure, hence, creating anisotropic structure.[150] Furthermore, Krintiras et al.. (2016) also found out that fibrous matrix produced from plant-protein ingredients exhibited anisotropic structure up to micrometer-scale, while for calcium caseinate, anisotropy was observed on a nanoscale.[151] The authors also showed that Couette cell technology can be scaled-up to a larger one without a barrier. Moreover, the structured matrix obtained with shear-flow technique is dependent on the temperature which the blends are deformed.[9] Thus, it is crucial to set temperature that will not affect the viscosity and protein conformation because it can negatively affect the phase separation.[152] Couette cell set up allows for more product yield, thereby demonstrating the potential of increasing the scale of production making it a promising texturing technique.

In vitro culture of animal meat using tissue engineering

Tissue engineering is also an innovative technology under investigation for culturing animal cells, which is useful for meat-like creation. This technique is based on stem cell technology whereby meat cells are isolated, ensuing isolated cells proliferation and differentiation into desired tissues *in vivo*.[153] Tissue engineering intends to produce a product with analogous organoleptic and nutritional qualities as conventional animal muscle.[69,154] During the tissue engineering process, myoblast cells are harvested from the animal skeletal muscle to culture muscle fibres.[155] Thereafter, standardized cell culture replicates these myoblasts with serum-supplemented medium containing key essential nutrients required for cell growth.[9] The production of normal development of muscle fibres most often uses an electric field or other means.

Potential scaffold materials for tissue engineering and culturing cell are micro-carriers, hydrogels, 3D bio-printing, fibre and porous scaffolds.[112] These technologies have emerged approximately in the last six decades for manufacturing skeletal muscle, bone, cartilage, fibrous and fat tissue. More recently, cell creation for recapitulating conventional meat texture parameters following products structuring in the absence of skeletal muscle tissue engineering has also been investigated.[156] Additionally, three-dimensional scaffolds surface morphology employed for tissue engineering has been improved by coating the 3D-printed scaffolds with electrospun nanofibers.[157] Besides, decellularized plant tissues, collagen, gelatin, synthetic polymers, and self-assembling peptides are materials that could substitute scaffolds in cultured meat.[112] Although, protein and peptide-based scaffolds are inexpensive, but they lack scaled sources appropriate for cultured meat. In summary, *in vitro* production of meat is possible with tissue engineering techniques. An effective culture of skeletal muscle would enable novel products with healthier or specialized products.

Antinutritional factors related to plant proteins

The demand for meat analogues is expected to rise at a considerable rate owing to the escalating global trend of healthy food consumption. Legumes are an excellent source of protein (20–35%), low in fat, and free from cholesterol. Apart from their nutritional benefits, the albumin and globulin fractions of legume proteins exhibit diverse functional properties, thus aiding in achieving the desired texture and functional characteristics during the development of meat analogue products.[158] However, legumes also contain various naturally occurring non-nutritive compounds referred to as antinutritional factors (ANFs), which can bind to proteins and reduce their absorption from the gastrointestinal tract. These compounds include trypsin inhibitors, tannins, and phytate.[159,160] Trypsin inhibitors can interfere with protein digestion leading to pancreatic hyperplasia/hypertrophy.[161] Meanwhile, phytate (myo-inositol hexaphosphate) has a strong affinity for metal ions such as iron, zinc, magnesium, and calcium, thus hindering the absorption of these minerals from the small intestine and potentially causing severe mineral ion deficiencies. Phytate has also been reported to bind with proteins and starch, resulting in reduced digestibility.[162] Tannins are water-soluble phenolic compounds that exist in hydrolyzable and condensed forms. Condensed tannins interfere with digestion by binding to proteins and minerals, whereas hydrolyzable tannins can cause liver and kidney toxicity.[163]

In the past, conventional techniques such as dehulling, soaking, cooking, and boiling were used to decrease the ANF contents of legumes and pulses. For instance, the trypsin inhibitory activity (TIA), phytic acid, and tannin contents of cowpeas (*Vigna unguiculata*) were reduced by 42%, 68%, and 79%, respectively, after soaking in water for 12 h and cooking for 30 min.[164] In another study, Wang et al. examined the effect of cooking on the ANF content of dry beans and chickpeas and found average reductions of 87% in the TIA content and 77% in the tannin content, while the phytic acid content remained unchanged.[165] However, overcooking has been reported to diminish the nutritional properties of pulses by reducing their essential amino acid contents.[165] Hefnawy reported that autoclaving and boiling were less effective in reducing the TIA, tannin, and phytic acid contents of lentils compared with microwave cooking.[166] By contrast, Luo & Xie reported that microwave cooking increased the phytic acid contents of green and white faba beans by 7.9% and 4.8%, respectively.[167] From this discussion, it is apparent that the efficacy of ANF reduction by conventional methods varies significantly and not every process is effective for decreasing ANF levels. Additionally, conventional processing methods have been reported to reduce the contents of lysine, tryptophan, and aromatic and sulfur-containing amino acids.[166] To address this issue, extrusion, as a high-temperature and short-time technique, may be a promising processing method for reducing the ANF content while improving the nutritional, textural, and sensory properties of pulse-derived ingredients during meat analogue production.

Effect of extrusion on ANF content and quality of plant proteins

Extrusion is a high-temperature and short-time thermomechanical process that induces various physicochemical changes, including a loss of the native protein structure, bond cleavage, binding, and aggregation.[168] It is also considered an effective method for nutrient retention, microorganism destruction, and ANF reduction compared with conventional cooking methods. The processing variables, mainly the barrel temperature, feed moisture, and screw speed, play key roles in achieving a significant reduction in the ANF contents of legume and pulse proteins. For instance, Broucke et al. Broucke et al. investigated the effects of low- and high-moisture extrusion conditions on the trypsin inhibitor content of a pea protein meat analogue and reported that both conditions afforded an 82% reduction in the trypsin inhibitor content at a barrel temperature of 140°C.[169] It is important to note that trypsin inhibitors are heat stable, such that a high barrel temperature (i.e., ≥140°C) is required for satisfactory removal. Accordingly, Rathod and Annapure reported that the TIA, phytic acid, and tannin contents of extruded lentils were remarkably reduced by 99.5%, 99.3%, and 98.8%, respectively, at a die temperature of 140–180°C and a screw speed of 150–250 rpm.[170] Likewise, the complete destruction of trypsin inhibitors in the common bean (*Phaselous vulgaris*) was reported after extrusion at 150–178°C with a feed moisture content of 23.7% and a screw speed of 414 rpm.[171] In another study, Nwabueze found that the trypsin inhibitor, phytate, and tannin contents of an African bread-fruit/corn/soy mixture were reduced by 91%, 44%, and 92%, respectively, when extruded at barrel temperatures of 120°C, 150°C (compression section), 170°C (metering section), and 150°C (die section) and screw speeds of 100, 120, 140, and 160 rpm.[172] For faba beans, extrusion cooking reduced the trypsin inhibitor, phytate, and tannin contents by 98.9%, 26.7%, and 54.4%, respectively, at a die temperature of 148–156°C, a feed moisture of 250 g/kg, and a screw speed of 100 rpm.[173] Finally, Brishti et al. developed texturized mung bean protein as a traditional animal meat alternative using extrusion processing at a barrel temperature of 144.57°C, a feed moisture of 49.33%, and a screw speed of 80.66 rpm and reported that the trypsin inhibitor, phytic acid, and tannin contents were reduced by 42.85%, 32.48%, and 6.11%, respectively.[74]

Although legumes have a high protein content, they also contain some major ANFs. If not removed or at least reduced, these may decrease the protein bioavailability such that the prepared foods are not nutritionally comparable to animal proteins. However, judicious regulation of the extrusion processing conditions, especially the barrel temperature, feed moisture, and screw speed, can significantly reduce the ANF contents of various legume and pulse proteins. It is therefore important to measure the ANF contents of plant protein-based meat analogues to ensure both food safety and protein bioavailability in the human body.[174]

Analytical methods for testing the quality attributes of meat analogues

Total protein content

From a nutritional perspective, PPBMAs should provide similar protein composition as the conventional meat since the main dietary purpose of consuming meat is “high quality protein”. It has been well established that PPBMAs containing up to 30% protein content can successfully replace meat.[123] Consequently, determination of the total protein content of meat analogues is required to fully understand the amount or concentration of proteins in the raw materials necessary for creation of the meat-like structure. Various protein assays based on both direct and indirect estimation have been employed to quantify the total protein content of meat analogues. Measuring the protein content based on the amino acid content of a sample is the direct method, whereas the indirect approach involves spectrophotometric evaluation of the nitrogen content.[175] Traditionally, the total protein content of the final meat analogues or raw materials is evaluated via the Kjeldahl method (e.g., AOAC 981.10 or 928.08), which involves a three-step approach comprising digestion, distillation, and titration.[176]

Another reliable analytical technique is the Dumas method (e.g., AOAC 992.15), which is based on sample combustion under an oxygen-rich atmosphere at 900–1300°C.[177] Technically, both methods can reveal the nitrogen (protein) concentration and different sample sizes using standards. Moreover, for both methods, the samples need to be homogenized prior to the measurement to enable reliable sampling because sample amounts are typically quite low.[176] SDS-PAGE is another technique used to profile the amounts and types of proteins present in the raw materials.[178] This electrophoretic technique can also unveil possible changes in the protein composition (e.g., protein degradation, crosslinking, structural modification), especially those occurring during extrusion owing to the harsh processing conditions.

Besides the protein content, Protein Digestibility Corrected Amino Acid Score (PDCAAS) is particularly important because protein bioavailability and bio-accessibility plays a major role in the overall nutrition. Structured PPBMAs should, therefore, present similar PDCAAS as conventional meat. Procedures for the determination of PDCAAS of several developed PPBMAs have been put forward by many researchers.

Microstructural characterization of PPBMAs

The texture of PPBMAs is an important attribute to evaluate their resemblance to conventional meat. For PPBMAs to successfully replace meat, developed products must possess a directional, layered, and fibrous arrangement. The microstructures and morphologies of PPBMAs have been studied using various microscopy techniques. Microscopic characterization allows visualization of the structure at different magnifications down to the nanoscale.[179] In particular, scanning electron microscopy (SEM) is commonly used to analyze the degree of dispersed phase deformation in the fibrous product. SEM analysis can produce surface images with nanoscale resolution (as low as ca. 0.5 nm). Notably, SEM can be effectively employed for the visualization of fibrous structures when the size range is not detected in other microscopy techniques. In recent years, SEM has been used to reveal a compact tightly connected network matrix in a soy protein isolate/pectin composite,[180] soya,[181] and fibrous layers in extruded lupin protein PPBMAs.[182]

Another imaging technique, confocal laser scanning microscopy (CLSM), is commonly applied to unveil microscopic features of the anisotropic structure of PPBMAs. This technique uses fluorescence to obtain two- and three-dimensional images of meat analogues.[149] CLSM makes it possible to detect elongated protein structural domains in plant-based meat analogues. It has also been applied in conjunction with nuclear magnetic resonance to acquire information on the moisture distribution and microstructural changes in meat and PPBMAs.[9] Furthermore, CLSM enables visualization of how deformation affects protein structural domains in meat analogues by analyzing shear- and non-shear-induced structuring.[179] Some typical SEM and CLSM images demonstrating the fiber formation in meat analogues under different conditions are given in Fig. 3a–f, respectively. Another microscopic technique is atomic force microscopy (AFM), which can be used to examine local three-dimensional structures on the nanometer scale. Conventionally, AFM is employed to quantify the tenderness or toughness of meat proteins, but it is not yet applicable to meat analogues. However, AFM tandem with infrared spectroscopy has been employed to obtain information regarding the distribution of lipid incorporated within the protein ingredients in a meat-like fibrous structure (Fig. 2).[95] Besides the aforementioned techniques, light microscopy can also be used to observe the fibrous network[10] while X-ray tomography allows visualization and quantification of the air in PPBMAs.[56] Overall, the major drawback of microscopic characterization techniques is that they require laborious sample preparation.

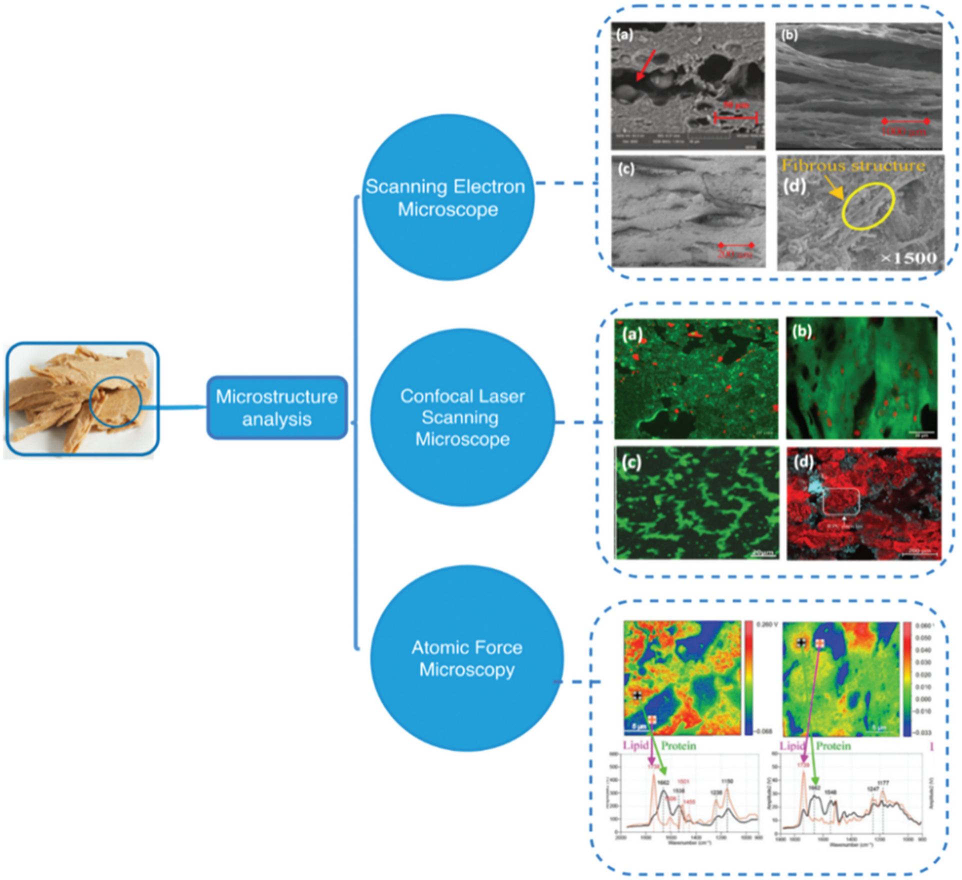


Figure 3. Micrographs of plant-based meat analogue using different microscopy techniques (a) extruded pea protein,[125] (b) structured soy-based using simple shear and heat in a couette Cell.[126] (c) concentrated soy protein isolate - pectin blend[162] (d) extruded pea protein-amylose/amylopectin blends.[59] CLSM micrographs of plant-based analogue samples. (a) Extruded wheat gluten with added oil at 800 rpm,[127] (b) protein structure of SPI-WG extrudates[128] (c) structured spi-sodium alginate blends,[129] (d) microstructure of the sheared products from rapeseed protein concentrate-wg at ratios.[122]

Viscoelasticity

Undoubtedly, the viscoelastic (i.e., both viscous and elastic) flow properties provide a useful insight into the deformability of the dispersed phase during structuring. Owing to the processing conditions (i.e., temperature, pressure, and concentration) used during structuring, predicting the emulsion dispersed phase deformability is complex. Hence, using a classical rheometer is not realistic.[9] To evaluate the viscoelastic properties of plasticized proteins, various rheometric systems have been proposed instead of a conventional rheometer, while imitating the process conditions in the course of structuring. The rheometric systems used include closed cavity rheometers, on-line slit die viscometers, and capillary die viscometers.[180] A closed cavity rheometer can ensure that parallel conditions in terms of pressure and temperature are used during structuring. Nonetheless, this rheometer is designed primarily to apply oscillatory shear to deduce the viscosity at low shear rates from $G''(w)$, linear viscoelastic functions, and $G'(w)$.[183] Previous studies have defined the behavior of PPBMAs produced from rapeseed,[184] wheat gluten,[183] and soy and pea proteins.[179]

On-line slit die viscometers that have known lengths are affixed at the end of the extruder and set to work at a constant flow rate. This is required to quantify the pressure gradient and then measure the mechanical/thermal stress profile using the Newtonian flow hypothesis.[180]

Capillary die viscometers are known to be the most precise technique for evaluating the viscosity of plasticized proteins; therefore, they are often employed for this analysis. Capillary die viscometers have been used to determine the melt viscosity for only non-Newtonian fluids.

In a recent study, food scientists used both rotational and capillary rheometers to provide insights into the viscoelastic properties of meat analogues, most especially for extrusion and 3D-bioprinting processes.[174] Furthermore, the shear cell technique can function as a rheometer besides being a structuring system because the torque values during cone rotation can be measured.[180] Nonetheless, the precision of torque values is insignificant.

Color

Color parameters are crucial to understand and compare how the texturing methods used for plant proteins influence the final color of PPBMAs. Different food colorants are typically added to fully mimic the distinctive red, white, or beige color of actual meat and meat products. To be effective as a good substitute to meat, the appearance (color) of PPBMAs must be similar to meat. As a food item, the visual appearance of PPBMAs is among the most important sensory aspects and therefore plays a crucial role in product acceptance. Moreover, as with other approaches, careful color and visual evaluation must be conducted for PPBMAs to ensure consistent results.[177] Generally, a colorimeter is used to obtain detailed information regarding the visual parameters of PPBMAs, with the results given according to the CIE L*a*b* system and D series illuminant (standard-D65).[176] Samples are illuminated with a standardized light beam by fitting the colorimeter with a pulsed xenon arc lamp. Photocells then collect the light reflected from the sample surface, which is used to estimate the coordinates in the color profiles. The reported result is standardized by calibrating the colorimeter using a white plate prior to quantification. The color quantifications are interpreted by using the CIELAB color space, in which L*, a*, and b* denote the lightness, redness/greenness, and blueness/yellowness values, respectively, of the test material.

In addition, several researchers have used a sensory evaluation approach in their studies to evaluate the overall appearance of PPBMAs. Gómez et al.[185] performed hedonic tests to examine the effects of cooking temperature and time on the color parameters of ready-to-eat PPBMAs. The hedonic score results were similar to the values for lightness and redness determined by colorimetry. Likewise, Sharima-Abdullah et al.[186] assessed the color and appearance of meatless nuggets prepared from chickpeas using hedonic tests. To obtain reliable colorimetric results, practical precautions should be taken during the color measurements. For example, real impressions of the visual appearance of the product should be acquired by digital photography and then harmonized with the L*a*b* results to help evaluate the appearance of the product.[176]

Sensory evaluation

Sensory characteristics, including the color, flavor, and mouthfeel, are crucial for convincing consumers and evaluating the market prospects prior to production. In the development of PPBMAs, a variety of precursors have been utilized to mimic aromas in the final product. Particularly, roast flavor or aroma is the most desired among several aromas created.[123] However, under high temperature and pressure during processing, complex chemical reactions occur which makes PPBMAs susceptible to off-flavors formation. Therefore, comprehension of the sensorial characteristic of the product is important. Instrumental analysis can be applied to evaluate the sensory characteristics of PPBMAs. Color measurements of meat analogues have been assessed using a color analyzer on the basis of UV – vis spectroscopy,[69] in which the absorbance was recorded at a wavelength of 510 nm. To determine flavor, the volatile compounds or profiled volatile constituents in the products can be quantified by gas chromatography – mass spectrometry, while non-volatile tastants can be detected by liquid chromatography – mass spectrometry. Meanwhile, the texture differences of PPBMAs can be assessed using either Warner – Bratzler shear force testing or a texture profile analyzer. Sensors based

Table 4. Different sensory evaluation methods of plant-based analogues.

Analogue ingredient	Sensory Testing	Sensory descriptors	Rating scale	References
SPI, WG, WS, carrageenan, and guar gum	Descriptive analysis	Elasticity, firmness, stickiness, roughness, compactness, juiciness, taste, and overall acceptance	Seven-point scoring test	[187]
Dry-fractionated pea protein, PPI, SPI, and oat protein	Descriptive analysis	Appearance, taste, odor, and texture	Eleven-point structured scale	[188]
TVP, methylcellulose, and seasoning	Affective analysis	Appearance, color, firmness, shape, and overall acceptability	Nine-point hedonic scale	[189]
Legumeat® Beef-Bean (defatted soy, bean, rice)	Consumer hedonic evaluation	Odor and color parameters	Seven-point hedonic	[185]
TVP and chickpea flour	Hedonic test	Appearance, aroma, colour, taste, texture, and overall acceptance	Nine-point hedonic scale	[186]
Faba beans protein concentrate, non-starch polysaccharides	Descriptive Analysis	Aromas, taste/flavor and texture	A 15 cm non-structured continuous line scale	[190]
Edible mushroom, SPI, and additives	Consumer acceptance test	Color, flavor, texture, mouthfeel, and overall acceptability	Nine-point hedonic scale	[135]

SPI = Soy protein isolates; WG = Wheat gluten; WS = Wheat starch; PPI = Pea protein isolate; TVP = Texturized vegetable protein.

on machine learning and artificial intelligence systems mimicking the human senses are also being developed to evaluate sensorial qualities.[69] Apart from instrumental analysis of sensory properties, panelists or consumers can help manufacturers to evaluate the overall characteristics of PPBMAs. Because the aim of producing meat alternatives is to mimic the quality parameters of actual meat, sensory evaluation is often used to evaluate their similarities.

Evaluation of the sensory characteristics of PPBMAs based on the sensory knowledge of consumers for conventional meat products is more crucial than using instrumental measurements. In particular, this helps to reveal some product features such as texture and flavor perception during consumption.[177] In the sensory studies of meat and PPBMAs, both descriptive analysis and affective testing sensory evaluation methods have been employed. In descriptive sensory testing, panelists are usually pre-trained in assessing meat analogues and are then asked to identify and rate given attributes (i.e., descriptors) that best fit the products on a predefined scale.[10] Various sensory attributes, including fibrousness, hardness/firmness, elasticity, juiciness, tenderness, crumbliness, and umami taste, are typically used in descriptive testing to characterize meat analogues.[149] Table 4 summarizes the different sensory evaluation methods for plant-based meat analogues. Overall, sensory testing serves as a valuable analytical tool to predict the sensory quality of PPBMAs, but the obtained data are very subjective and may not be an optimal representation of the sensory impression of the product.

Technological and consumer perception/acceptability-related challenges and opportunities

Meat analogue/meatless meat products are transitioning from niche products to commonplace ones, among which PPBMAs in particular are witnessing remarkable economic growth.[142] PPBMAs are intended to mimic the sensory features of conventional animal-based meat products, such as their outward appearance/color, flavor, and fibrous texture.[123] Recently, with “Better for you” and “Better for the planet” trends rising in popularity, PPBMAs have been advocated as feasible protein substitutes, contributing a plethora of beneficial characteristics for human health as well as potential opportunities for reducing meat consumption.[70,191] PPBMAs are protein-rich products promoted as possessing a well-balanced essential amino acid composition and being low in saturated fats and free of cholesterol, with similar nutritional values to meat-based products, and their consumption is reported to be potentially linked to various health benefits, whereas several health issues are believed to be strongly associated with excessive consumption of food products based on real meat.[69,192,193]

Considering food safety and consumer health as primary concerns, plant-based meat analogues have emerged as replacements/substitutes for conventional animal-based meat products.[194] Nevertheless, several technological and consumer-related challenges (Fig. 4) have affected this momentum by directly influencing consumer acceptability and consumption behavior for plant-based meat analogues.[195]

Besides the production constraints, economic challenges are also hindering the greater acceptance of PPBMAs, which are considerably more expensive than traditional meat-derived food products owing to the extensive processing and high amounts of ingredients/additives needed.[11] Therefore, additional innovative procedures such as optimized processing conditions and less expensive additives are urgently required to reduce production costs while maintaining product quality. The influence of post-production handling and packaging conditions on the shelf life, microbial safety, and sensorial characteristics of PPBMA-based products should also be monitored.[123,196] Regulations and legislation, in terms of ingredient labels, product naming, and new product marketing, have been raised as another critical issue for plant-based meat analogues.

Safety and consumer acceptance of PPBMAs

Many health-conscious consumers, particularly those following vegan, vegetarian, and flexitarian diets, have in the last few years been looking for safer and healthier food products, thereby shifting consumption preferences toward plant-based meat analogues.[196] Multiple factors, including pricing, outward appearance, appropriateness, quality, security, socio-cultural, singular, and commercial/industrial aspects, have been found to affect the willingness of consumers to purchase PPBMA-based products[185] (Fig. 4). By endorsing the health benefits and sustainability of PPBMA-based

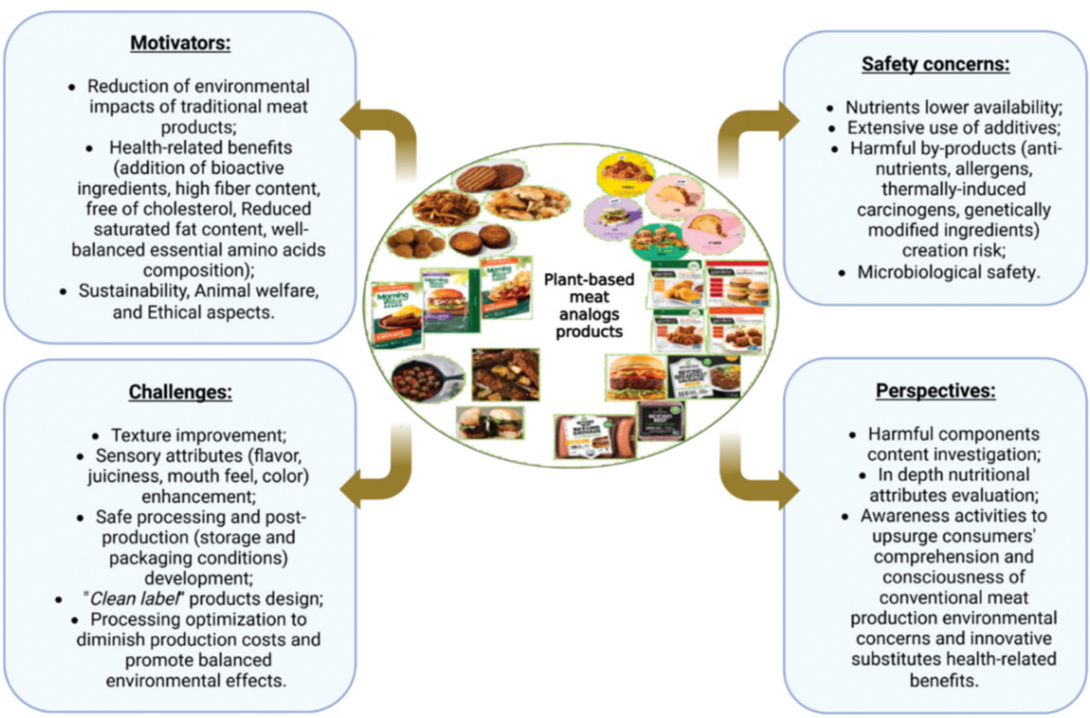


Figure 4. Motivators, challenges, safety concerns, and future perspectives allied with plant-based meat analogs products.

products compared with those originating from animal sources, alongside appropriate marketing and consumer consciousness campaigns, the media are partially responsible for shifting the dietary preferences of consumers.[197] In a recent study, Sun et al.[113] reported that PPBMAs are particularly accepted by four principal consumer segments, including individuals aiming to reduce their consumption of meat-based products and seeking healthier and well-coordinated nutritional diets, individuals aware of animal well-being, sustainability, apart from the ethical aspects, individuals who are emphasizing on suitability/satisfaction and price, and individuals who are geared toward extravagance and modernization/novelty.[113] The consumer acceptability of PPBMA consumption has also been reported to be additionally influenced by demographic determinants including age, gender, revenue, educational status, and geographic area.[198] It has been well established that the decision of consumers to purchase plant-based meat analogues is associated with environmental concerns and convenience for young consumers (less than 34 years old) and product familiarity and taste for older consumers (45–59 years old).[198,199] In addition, the increased consumption/purchasing of PPBMAs was reported to be day-by-day further frequent among low-income consumer groups.[198] Furthermore, females aged 18–34 years with higher levels of education were reported to exhibit greater awareness of the drawbacks of animal-based meat products and the associated environmental issues, thus encouraging them to consume/purchase PPBMAs; however, females aged over 55 years were primarily concerned with issues related to their health.[199]

Undeniably, an increased number of consumers are in search of sustainable food products and eco-friendly processing approaches, inspiring them to switch to vegetarian diets or reduce their meat consumption, decreasing the demand for natural resources and corresponding pollution emissions.[185] Nonetheless, PPBMAs evolving popularity and markets are still fronting consumers' acceptability and preferences doubts challenges.

Future perspectives in PPBMA research and commercialization

Although PPBMA-based products are increasingly gaining acceptance from consumers, this remains difficult, which is primarily attributable to a lack of consumer familiarity with these novel products. Repeated exposure via education and appropriate advertising/marketing strategies could promote the likelihood of consumers purchasing PPBMAs.[200]

Compared with initial attempts to imitate the organoleptic and nutritional characteristics of real meat, it is undeniable that the taste and texture of innovative plant-based meat analogues have been substantially improved, which can be considered great technological achievements by the food industry and scientists. However, additional development efforts and marketing of PPBMA-based products can be expected, ultimately promoting further improvements in the popularity and economic value of PPBMAs in the upcoming decades.[131,201] Moreover, other key aspects to be considered are the quality characteristics and security of PPBMA-based products, although such products are commonly recognized as safer than animal-based meat, specifically in terms of the impact of intensive production processes on the quality features of the final product.[69] The development of effective approaches to boost the shelf life and guarantee the health and microbiological security attributes of PPBMA-based products is also considered of great importance. Among the most reported techniques, the addition of naturally occurring antioxidants and functional ingredients has emerged as an effective and interesting approach.[113,123,202] However, with the increase in the number of purchasers seeking "clean label" products, creative techniques for the preservation of meat analogue-based products are expected. Specific consideration needs to be given to evaluate the risk of elevated temperatures generating undesirable byproducts during PPBMA processing. As such, low-temperature processes such as super-chilling, immersion vacuum cooling, and freezing (ultra-rapid, hydro-fluidization, impingement, electrostatic-assisted, pressure-shift, etc.) and techniques not involving heat treatment, such as acidic electrolyzed water associated with high hydrostatic pressure and non-thermal plasma, and their applicability to the preservation of plant-based meat analogues need to be investigated.[193]

Consumer acceptance and purchasing of innovative meat alternatives are crucial to the successful development of PPBMA-based products with high popularity and economic value. Accordingly, future endeavors by modern food producers and scientists ought to concentrate on technological challenges as well as nutritional value enhancement and process optimization to reduce production costs and promote balanced environmental effects.[189,203] Because of emotional attachment (pleasure and enjoyment of eating meat) and/or a fear of consuming unfamiliar food products, modern foods can be less attractive and may even be rejected by consumers who prefer to keep their existing dietary behavior. Consequently, it is essential to promote a greater awareness and understanding of plant-based meat analogues and the environmental issues associated with conventional meat production.[197]

Conclusion

The increasing concerns about animal welfare, environmental problems, ethical considerations, and health worries have led to the rising popularity of meat analogues. However, they encounter certain limitations and challenges that must be resolved to ensure their widespread acceptance and consumption. It is noteworthy that imitating the comprehensive sensory experience of real meat, particularly in relation to texture, moisture, and flavor, remains a challenging task. There exists a pressing need to enhance their nutritional profile by improving protein functionality and quality, decreasing salt content, and incorporating beneficial elements such as bioactive compounds, minerals, and vitamins. Meat analogues are confronted with technical and regulatory hurdles that restrict their production and distribution, and they must adhere to food safety standards and labeling regulations in various nations. However, these alternatives to conventional meat exhibit a promising future as sustainable and nutritious options in light of burgeoning population, limited resources, and environmental obstacles. Furthermore, their potential for enhancing food security and diversity by providing a wider range of options that suit various dietary needs and preferences is immense. Research and development of meat analogues have gained increasing interest, with particular focus on enhancing the sensory and nutritional attributes of their products by utilizing innovative ingredients and processing techniques. Employing effective marketing strategies and communication channels to heighten consumer awareness and education about the benefits and safety of meat analogues could be advantageous for promoting their adoption and consumption.

Abbreviations

PPI	Pea protein isolate
MPI	Mung bean protein isolate
MF	Mung bean flour
DPF	Defatted peanut flour
PSPI	Pumpkin seed protein isolate
PSF	Pumpkin seed flour
RPI	Rice protein isolate
SPI	Soy protein isolate
HPC	Hemp protein concentrate
WG	Wheat gluten
PPBMAs	Plant protein-based meat analogues
ANFs	Antinutritional factors
TIA	Trypsin inhibitory activity
SEM	Scanning electron microscopy
CLSM	Confocal laser scanning microscopy
AFM	Atomic force microscopy
HMMA	High-moisture meat analogue
HME	High-moisture extrusion
TVP	Texturized vegetable protein
PDCAAS	Protein Digestibility Corrected Amino Acid Score

Acknowledgments

The authors would like to thank the United Arab Emirates University for funding this study through Asian University Alliance grant with fund code 12F002.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The work was supported by the United Arab Emirates University [12F002].

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