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Alternative proteins; A path to sustainable diets and environment

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ABSTRACT

With a growing global population and the resulting pressure on natural resources, the supply of high-value protein has become increasingly limited. The rise of environmental and ethical concerns has led to the emergence of meat analogues as a credible alternative to traditional animal-derived meat. Growing demand for plant-based protein sources has gained attention as viable alternatives to conventional animal proteins. This article reviews commercially available plant proteins for meat replacement and evaluates recent research on producing meat analogues, highlighting their advantages and limitations. Beyond production, an examination of the physicochemical, textural, and structural attributes of the meat alternatives is conducted, highlighting the improvements made in achieving sensory and nutritional parallels with animal-derived meat. Furthermore, this article explores the current commercial applications of meat alternatives, highlighting the challenges faced in their widespread adoption and suggesting future research directions. The comparison of the environmental impacts of plant proteins and animal proteins is also presented. The ultimate goal is to develop meat substitutes that closely mimic the sensory, nutritional, and aesthetic qualities of real meat. Despite promising innovations in processing technologies, challenges remain that researchers are actively addressing to close the gap between plant-based meat analogues and animal-derived counterparts.

1. Introduction

Proteins constitute essential elements within the human dietary framework, serving as key agents in cellular restoration, immune regulation, and the maintenance of muscular integrity (Nasrabadi et al., 2021). The increasing demand for protein, driven by global population expansion, urbanization, and economic prosperity, has placed extreme stress upon the food industry, requiring increased focus on both food safety and nutritional security (Zhang et al., 2023). Animal-derived proteins, serving as the main protein source, have been criticized for their role in diminishing animal welfare, contributing to various health concerns and diseases, and negatively impacting the environment (Nasrabadi et al., 2021). Animal protein production is projected to rise by approximately 50–73% by 2050 to support the needs of a rapidly increasing and economically advancing global population (Chiang et al.,

2021). The World Health Organization (WHO) has highlighted the pressing demand to replace animal-derived proteins with sustainable protein alternatives (Kumar et al., 2023). Nutrition experts and researchers have displayed significant interest in sustainable protein sources, notably plant-based proteins like wheat gluten (WG), soy and peas proteins (Schreuders et al., 2021). Plant-based proteins offer numerous advantages when compared to animal-derived proteins. These benefits include enhanced sustainability, reduced restrictions based on religious and cultural dietary practices, enhanced animal welfare considerations, diminished susceptibility to contamination and infection, and increased cost-effectiveness (Jafari et al., 2022). Numerous investigations within the domain of food research have been undertaken to explore the feasibility of substituting animal-based proteins with their plant-based counterparts (Tyndall et al., 2022). Researchers have engineered meat analogues utilizing a range of plant protein sources,

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including WG (Chiang et al., 2021), soybeans (Cornet et al., 2020), pea (Osen et al., 2014), peanut (Zhang et al., 2020a), and mung bean (Brishti et al., 2021). It is important to note that plant-based proteins are not without their drawbacks, despite their numerous benefits. These include lacks in certain essential amino acids, the existence of antinutritional factors (ANFs), and limitations in providing the same textural and sensory properties as animal proteins. Addressing these issues is essential to promote plant-based proteins' wider use in food applications (Craddock et al., 2021). A review of plant-based diets' impact on human health and climate change indicates that adopting a mostly plant-based diet is unlikely to adversely affect nutrient status. However, this outcome is highly dependent upon the specific foods consumed in the diet (Meulenbroeks et al., 2021). The creation of plant based meat analogues encompasses a diverse array of techniques, primarily involving the manipulation and reformation of protein fibres to replicate the textures and structures akin to animal-derived proteins (Singh and Sit, 2022). Over the last few decades, several technological advancements have significantly contributed to the evolution of plant protein reconstruction methods. These innovations encompass spinner technology, extrusion technology, shell-cell technology, and freeze-casting technology, with each offering distinct advantages and inherent limitations (Sha and Xiong, 2020). Recent scholarly reviews have examined plant-based meat alternatives, with one prominent investigation by Van der Weele et al. (2019) introducing a comprehensive conceptual framework for evaluating the nutritional consequences and potential sustainability advantages of diverse meat alternatives, both plant-based and non-plant-based. The authors highlighted a significant gap in existing literature, particularly regarding plant-based meat alternatives that exhibit high sustainability potential, especially those derived from pulse crops (Van der Weele et al., 2019).

In the field of plant-based proteins and meat alternatives, our study stands out for its comprehensive approach and innovative insights. We offer a comprehensive comparative analysis of the environmental impacts of plant proteins versus animal proteins, highlighting the sustainability benefits of plant-derived alternatives. Additionally, we emphasize technological advancements driving plant protein reconstruction methods, highlighting practical challenges and breakthroughs shaping the future of plant-based meat alternatives. Our review also includes a detailed analysis of consumer acceptance and adoption, emphasizing the critical role of market dynamics and preferences. Lastly, we provide insightful recommendations for future research, aiming to enhance the development of superior plant-based meat alternatives. Through these contributions, we aim to advance understanding and accelerate progress in sustainable food systems.

2. Protein sources in meat alternatives

Meat is a fundamental part of many diets worldwide and a staple in numerous cuisines. It is highly nutritious, offering substantial amounts of protein and a rich array of macro- and micronutrients. These include iron, zinc, vitamins B1, B12, A, D, niacin, and proteins, contributing to its high nutritional value (Singh and Sit, 2022). However, primary sources of animal protein such as processed meat, unprocessed red meat (beef, pork, mutton), poultry, and fish can influence the risk of major chronic diseases in various ways (Zhong et al., 2022). On the other hand, global population growth has increased the demand for protein sources, increasing the pressure on conventional animal agriculture. This growing emphasis on alternative animal protein sources has arisen primarily from growing environmental concerns associated with traditional livestock farming practices.

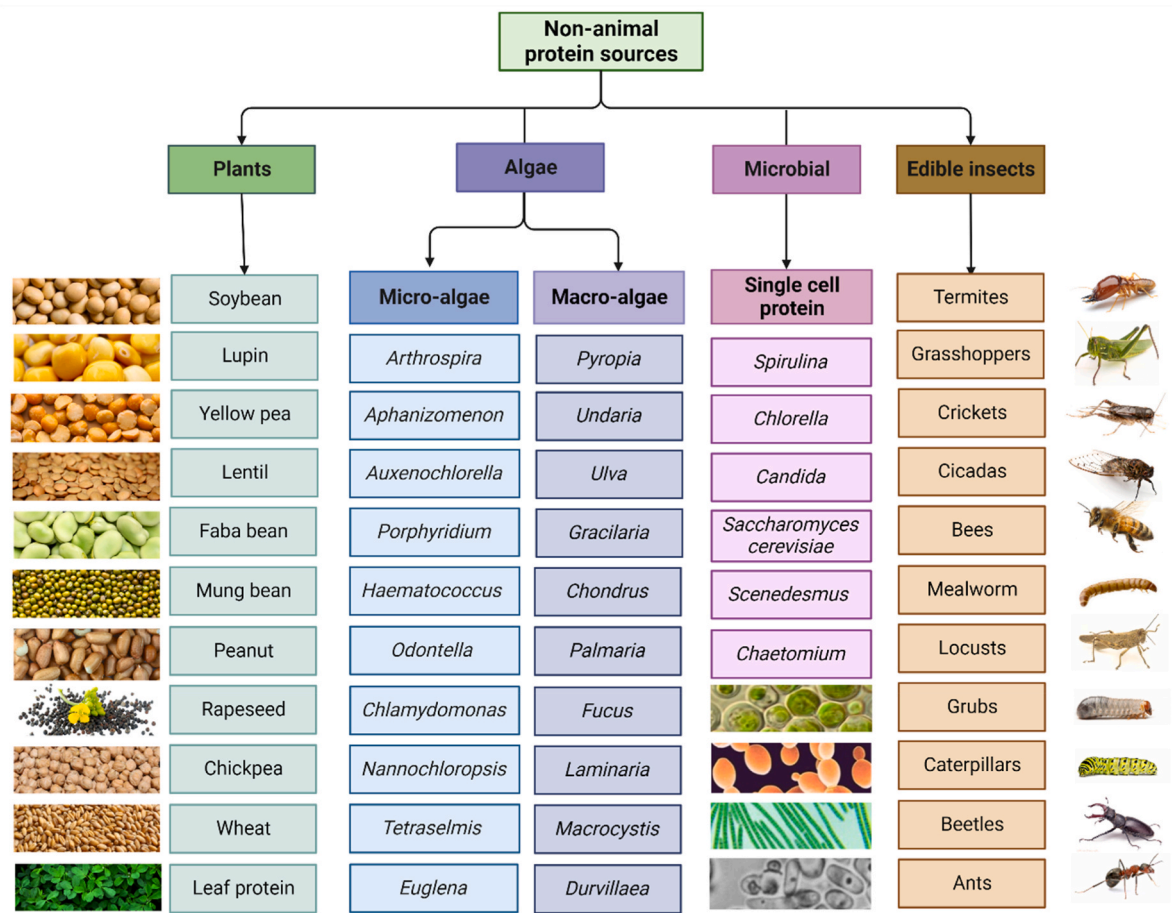


Fig. 1. Potential non-animal protein sources for production of meat alternatives.

Alternative sources encompass a diverse array of options, including insects, algae, fungal, and plant-based proteins (Fig. 1). Some examples of meat alternative sources and their compositions compared to the conventional meat sources are also presented in Table 1. Due to the growing demand for protein, these alternative sources offer a more sustainable and efficient means of meeting this demand while also mitigating the environmental impact of conventional livestock farming. Despite being relatively unfamiliar compared to traditional animal products, their acceptance is steadily growing as consumers become more aware of their numerous benefits (Floret et al., 2023). Each protein source presents unique challenges that are addressed through a range of potential solutions, such as optimizing cultivation conditions, enhancing sensory characteristics, and improving nutritional profiles. In the following sections, various alternatives to meat-derived protein are discussed.

2.1. Fungal-derived proteins

Fungal-based proteins are gaining attention for their high-quality nutrients and efficient production processes, which do not rely on traditional agriculture or weather conditions (Wang et al., 2023a). Fungal protein production has a significantly lower environmental

impact than conventional meat, with a carbon footprint 10 times lower than beef and 4 times lower than chicken (Majumder et al., 2024). Furthermore, fungi can transform various industrial and agricultural wastes into valuable protein compounds, supporting a circular economy (Wang et al., 2023a). This positions fungal protein as a promising new source of nutrition. Fungi can be categorized into three primary groups based on their life history: unicellular fungi, multicellular fungi, and macro filamentous fungi. Yeasts, molds, and macrofungi, which are the most common examples, have long been incorporated into human diets (De Sousa et al., 2024).

Fungi biomass contain a protein content ranging from 10 to 63%, along with significant amounts of dietary fiber, essential amino acids, vitamins B and D, minerals, and bioactive polysaccharides. Certain fungi are notably rich in sulfur-containing amino acids, which provide a meaty flavor (Perez-Montes et al., 2021; Mingyi et al., 2019). The Food and Drug Administration has granted Generally Recognized as Safe (GRAS) status to certain fungal species, including *Fusarium venenatum*, *Monascus purpureus*, *Aspergillus oryzae*, *Neurospora intermedia*, *Rhizopus oryzae*, and *Paradendryphiella salina*. In addition to mushrooms, mycoprotein, derived from filamentous fungi, is an excellent meat alternative, offering nutritional benefits and additional health advantages over traditional meat. It functions as a prebiotic, antioxidant, and regulator of blood

Table 1

Compositions of non-animal sources for production of meat analogues compared to the conventional meat sources.

Meat and meat alternatives sources	Main compositions (%)						Other compositions	Reference
	Protein	Carbohydrate	Lipid	Ash	Fibre	Moisture		
Macroalgae <i>Rugulopteryx Okamurae</i>	12.2	60.4	17.3	11.3	ND	6.75	Vitamins, minerals, omega-3 fatty acids, peptides, polyphenols, sulfur-rich substance, antinutritional factors including saponin, phytic acid, and tannins	Cebrián-Lloret et al. (2024)
Microalgae <i>Chlorella vulgaris</i>	38.85	28.35	24.50	7.58	ND	ND		Koochi et al. (2023)
Microalgae <i>Spirulina</i>	64.2	18.7	7.2	9.9	ND	ND		Villaro et al. (2023)
Adult Cicada insect (<i>Meimuna opalifera</i> Walker)	56.35	8.38	13.37	16.87	16.87	ND	Omega 3, 6, and 9 fatty acids, vitamins (B, A, D, E, K, and C), minerals (Fe, Zn, Ca, Na, K, Mn, and P), ANFs like tannin, oxalate, and phytate	Li et al. (2024)
House Cricket (<i>Acheta domesticus</i>)	71.7	1.6	10.4	5.4	4.6	6.3		Udomsil et al. (2019)
Field Cricket (<i>Gryllus bimaculatus</i>)	60.7	0.1	23.4	2.8	10.0	3.0		
Soybeans (Yellow and black)	38.08–41.24	17.67–31.38	6.46–10.86	4.65–5.31	ND	4.55–8.77	Minerals (Fe, Ca, and Zn), vitamin B group, ANFs such as trypsin inhibitor	Anjum et al. (2022)
Lupin flour (<i>Lupinus angustifolius</i> L.)	35.77	4.59	6.05	3.57	13.60		Minerals (Fe, K, Mn, and Zn), ANFs such as non-hydrolysable oligosaccharides, phytic acid, alkaloids, and other secondary metabolites	Olukomaiya et al. (2020)
Yellow split pea flour (<i>Pisum sativum</i> L.)	22.10	65.99	1.21	2.67	1.10	10.85	Minerals (Fe, Se, and Zn), vitamin B group, ANFs such as tannins, phytate, and trypsin inhibitors	Fahmi (2022)
Lentils (<i>Lens culinaris</i> Medik.)	18.21–26.42	69.60–77.03	0.74–1.91	2.07–3.91	ND	ND	Minerals (Fe, Mn and Zn), vitamins B group and C, ANFs such as tannins, phytate, and trypsin inhibitors	De Angelis et al. (2020)
Faba beans (<i>Vicia faba</i> L.)	22.39–31.07	63.85–71.65	0.99–2.20	3.11–4.46	ND	ND	Minerals (K, Fe, Mn), vitamins B group and C, ANFs such as lectins, trypsin inhibitors, and tannins	De Angelis et al. (2021)
Wheat grains (<i>Triticum aestivum</i> , and <i>monococcum</i>)	12.83–18.95	56.15–70.44	1.77–2.35	1.77–2.47	ND	11.52–13.24	Minerals (K, Fe, Mn) and vitamin B group	Golea et al. (2023)
Mycoprotein	40–45	3.0	2.9	ND	6.0	ND	Cholesterol, minerals (Zn), vitamins B group and D	Ahmad et al. (2022)
Beef	17.0–20.2	ND	3.8–13.2	1.6–2.7	ND	68.2–73.6	Minerals (Fe, Zn, Sn, P, Mn, and K), vitamin B group	Yeh et al. (2018)
Poultry	13.1–16.3	5.8–12.6	2.2–2.6	0.9–1.1	ND	71.0–75.6	Cholesterol, minerals (Fe, Mn, Na, and Ca) and vitamins such as B6, B12, and C	Chepkemol et al. (2017)
Fish	13.1–20.3	ND	1.9–7.6	2.8–7.0	ND	65.5–76.8	Omega-3 fatty acids, minerals (Ca, P, Fe, Zn, I, Mn, and K) and vitamins such as D and B2	Sumi et al. (2023)
Mutton	21.6	ND	4.9	2.6	ND	71.9	Cholesterol, minerals (Fe, Na, Mn, and Ca) and vitamins such as B6 and B12	Skele et al. (2024)

ND: not determined.

cholesterol and glucose levels. *Fusarium venenatum* ATCC 2684, developed in the 1960s, is the primary strain used for mycoprotein cultivation. In 1984, the UK's Ministry of Agriculture, Fisheries and Food authorized the sale of mycoprotein as a food protein, and it is now available across all EU member states (Whittaker et al., 2020). The Quorn™ brand prominently features mycoprotein in its vegan and vegetarian products. Production methods include submerged fermentation, solid-state fermentation, and surface culture, with the yield varying based on the microorganism or substrate used. Research indicates that submerged fermentation produces higher yields and greater nutritional benefits (Majumder et al., 2024). However, there are challenges in using fungi as protein alternatives. Many fungi produce toxic secondary metabolites during cultivation, making the protein unsuitable for human consumption (Zhang et al., 2024). Besides, lower protein content, potential for nausea, vomiting, and allergic reactions are the other challenges necessitating the careful evaluation of novel fungal proteins for use in meat alternatives (Majumder et al., 2024).

2.2. Algae-derived proteins

Algal biomass is considered a viable alternative to animal-derived proteins, offering several advantages. These benefits include a faster growth rate, minimal water usage, no need for fertile land, zero carbon emissions, and the ability to produce a wide range of bioactive compounds (Geada et al., 2021). Algae can exist in different forms, including colonial, unicellular, filamentous, or as simple tissues (Guiry, 2012), and can be categorized as micro- or macro-algae. Microalgae, such as *Arthrospira* and *Chlorella*, are rich in essential amino acids and offer health benefits comparable to animal proteins (Koyande et al., 2019). Some less common microalgae, like *Odontella*, *Haematococcus*, *Tetraselmis*, and *Euglena*, are listed as novel foods by the European union (EU) (Lafarga, 2019). Macroalgae, or seaweed, have been part of the human diet for a long time, and therefore, more varieties have been approved by the European Food and Safety Authority (EFSA) (Banach et al., 2020). Examples include *Porphyra tenera*, *Ulva lactuca*, *Undaria pinnatifida*, *Chondrus crispus*, *Palmaria palmata*, and *Fucus serratus* which contain protein contents of 44, 29, 29, 20, 19, and 17%, respectively (Orkesterjournalen, 2018). Proteins derived from dried microalgae cells along with certain bacteria and fungi, are commonly referred to as single cell protein (SCP). These SCPs serve as protein supplements in both food and animal feed (Fig. 1) (Geada et al., 2021). Microalgae typically have a high protein content (over 70%), while seaweeds generally have lower protein content (9–22%), although specific red seaweed species may have protein content reaching up to 47% (Bleakley and Hayes, 2017). Additionally, algae contain essential nutrients like dietary fibres, vitamins, minerals, and omega-3 fatty acids (Baune et al., 2022). Therefore, the rise of commercial algae-based foods aligns with consumers' preferences for healthy, nutritious, ecological, and vegan products.

In addition to their potential as an alternative protein source, algae proteins can produce bioactive peptides which have positive effects on health, such as antioxidant, anti-proliferative, anti-inflammatory, anti-hypertensive, anti-diabetic, anti-atherosclerotic, anti-coagulant, and antimicrobial properties (Pimentel et al., 2019). However, there are some challenges in using algae as a food product or ingredient. These include the strong and undesirable aroma and colour related to pigments and sulfur-rich substance, as well as lipid-based volatiles, which negatively affect the sensory qualities of the final product (Lafarga, 2019). In addition, the presence of antinutritional factors (ANFs) in algal biomass is a critical point which should be addressed before industrial utilization. In this regard, ANFs including saponin, phytic acid, and tannins has been recognized in *Chlorella pyrenoidosa* biomass (Chen et al., 2022a). Furthermore, algae often have lower digestibility when consumed in their raw form due to the composition of the cell walls, which typically contain high amounts of dietary fibres and potentially polyphenols.

Extraction of the algae protein can improve the digestibility of algal protein eliminating the cell walls, which can in parallel affect the

production cost (Grossmann et al., 2020). However, when dealing with seaweeds, the extraction process can be hindered by the presence of high viscosity cell wall polysaccharides like carrageenan, alginate, or agar which are obstacles to separate and recover the protein fractions. It is important to design a sustainable recovery procedure that considers the characteristics and nature of the algae wall. To facilitate extraction, it is usually necessary to use disruption techniques that break the cell membranes and allow access to the internal components. The choice of extraction conditions depends on the desired objective since it directly affects protein bioavailability, functionality, and organoleptic properties (Bleakley and Hayes, 2017). Traditional protein extraction methods rely on physical processes like mechanical disintegration and non-mechanical extraction. However, emerging methods such as ultrasonication, ohmic heating, pulsed electric fields, and microwaves are being used to address issues like time consumption and loss of protein integrity. These methods can be combined with disruption techniques for protein recovery and concentration (Geada et al., 2021).

The manifold applications of algae protein extend to sectors such as food and beverage, animal feed, cosmetics, and pharmaceuticals, each poised to harness the nutritional merits it offers. Progressive technological advancements and ongoing research endeavours are driving an upswing in algae protein utilization, thereby underpinning the journey toward a more sustainable future (Bleakley and Hayes, 2017). In recent years, the compelling combination of high protein content and exceptional nutrient density has positioned algae as an appealing substitute for meat. The versatility of algae proteins extends to a diverse range of meat alternatives, encompassing options like burgers, sausages, and various other meat alternatives products. Furthermore, for consumers pursuing a meat-like gastronomic experience while maintaining an eco-conscious stance, certain algae species adeptly emulate the texture and flavour profile of meat (Geada et al., 2021). Over the past few years, spirulina has experienced a surge in popularity owing to its rich protein content and the abundance of essential vitamins and minerals. Spirulina, a type of blue-green algae, has found its way into a wide array of products such as snacks, bars, and non-animal milks. Notably, the integration of algae protein into meat products like sausages and burgers revealed that the inclusion of algae protein not only enhanced the nutritional profile of the meat products but also contributed to a reduction in their environmental footprint (Yucetepe et al., 2019). Additionally, the feasibility of employing algae-based proteins as a scalable and sustainable substitute for meat-derived proteins for human consumption was substantiated on a broad scale (Joniver et al., 2021). Collectively, the traditional consumption of meat is fraught with environmental and health challenges that can be mitigated through the adoption of algae-based proteins as a meat alternative. With ongoing advancements in research and technology, algae proteins will probably persist in their ascent to prominence as a sustainable and nutritive dietary choice.

2.3. Insect-based protein

Insect protein is rapidly gaining popularity as a viable alternative to conventional meat sources. Several factors determine the chemical composition of insects, including the species, season, their age, location, and the diet. Accordingly, insects, on average, contain approximately 40–70% of proteins and 10–60% of fat, omega 3, 6, and 9 fatty acids. They also contain various vitamins (B group, A, D, E, K, and C) and minerals (such as iron, zinc, calcium, sodium, potassium, manganese, and phosphorus), similar essential amino acids to what is found in beef and soybeans (Gorbunova and Zakharov, 2021). In this regard, termites (*Isoptera*) have a protein content of 34% (dry basis), while grasshoppers, crickets, and locusts (*Orthoptera*) have protein content of around 61%. The most abundant amino acids in insect protein are phenylalanine, leucine, valine, and tyrosine, although phosphorus amino acids and tryptophan are less common. More especially, during the nymph stage of insect development, all amino acids are abundantly present (Rumpold

and Schlüter, 2013). Overall, the amino acid profile of insect proteins aligns with the recommendations of the WHO. Although Insect proteins are more digestible (76–98%) compared to certain plant proteins like lentils and peanuts (52%), but slightly less digestible than beef and eggs (100%) (Gravel and Doyen, 2020). Besides, certain insects, like the mulberry silkworm and silkworm (*Bombyx mori* L.), have specific health benefits such as reducing hypercholesterolemia/atherosclerosis and acting as an antidiabetic, respectively (Jo and Tojo, 2019).

The adoption of insect protein as a meat alternative presents several advantages. In comparison to traditional meat sources, insects provide a nutritionally rich alternative, featuring protein, fibre, and a host of vital nutrients (Akhtar and Isman, 2018). Furthermore, they contribute to environmental sustainability by producing fewer greenhouse gas emissions and generating minimal waste. The production of insects is also relatively cost-effective, requiring less infrastructure and fewer resources than conventional livestock farming. Importantly, insects have a longstanding history of consumption as a food source worldwide (Berezina and Hubert, 2019). Researchers have conducted comparative studies between insect and beef protein production, revealing a substantial reduction in greenhouse gas emissions and water usage in favour of insect protein (Oonincx and De Boer, 2012).

While insect protein holds promise as a meat substitute, it does come with certain challenges. Legal restrictions in some countries may prohibit the consumption of insects, and cultural barriers can impede their acceptance (Gorbunova and Zakharov, 2021). Additionally, concerns related to food safety and hygiene surround insect production and processing. Besides, cross-reactive proteins in some edible insects can cause allergenicity such as carmine produced from the female cochineal insects as food dye. It is also necessary to pay attention to the unknown potential allergens in edible insects. Many phytophagous insects retain ANFs at high levels. Insects such as winged termites (*Macrotermes bellicosus*) and rhinoceros beetles (*Oryctes monoceros* and *Oryctes boas*) have been found to contain ANFs like tannin, oxalate, and phytate. These substances can disrupt metabolic processes, decrease nutrient bioavailability, and inhibit digestive enzymes. Phytate and oxalates can form insoluble compounds with metallic ions, reducing their absorption. Additionally, oxalates may cause irritation and swelling in the mouth and throat. However, the nutritional and antinutritional content of edible insects is largely influenced by their environment and diet (Idowu et al., 2019). Several approaches are utilised to process insects as a food ingredient such as various drying techniques (sun, freeze, oven, or microwave), ultrasound extraction, and cold atmospheric pressure plasma processing. In a study conducted by Cho and Ryu (2021), the authors examined how varying levels of mealworm larva (0%, 15%, and 30%) and different extrusion parameters affected the characteristics of extruded meat analogues. A noticeable alteration in the extruded meat analogues was observed as mealworm larva content increased. A higher concentration of mealworm larva reduced the integrity index, textures profile analysis scores, and oxidative activity of extruded meat analogues. In contrast, greater mealworm content increased water retention, nitrogen solubility index, protein digestibility, and radical scavenging activity (Cho and Ryu, 2021). Another study highlighted the significance of insect species as abundant sources of protein, vitamins, and minerals, affirming the nutritional value of insects as a sustainable food source for humans (Haber et al., 2019).

Consumer attitudes toward insect consumption, including potential feelings of disgust and food neophobia, are pivotal in the broader acceptance of insect-based proteins. Food neophobia, defined as the reluctance to eat new foods, often manifests when encountering unfamiliar culinary experiences such as insects. However, as society evolves and becomes more receptive to exploring novel food options, these initial hesitations can be overcome. Educational efforts and exposure to insect-based products in various formats can help mitigate food neophobia by demonstrating insects' nutritional and environmental benefits (Gravel and Doyen, 2020). Vegetarians and vegans, driven by ethical and environmental concerns, may find insect proteins appealing if they

align with their values regarding animal welfare and environmental sustainability. Their willingness to consider insect-based alternatives would depend on the assurance of humane insect farming practices and minimal environmental impact (Elorinne et al., 2019). Addressing food neophobia and fostering a positive perception of insect consumption requires a multifaceted approach, encompassing education, product innovation, and assurances regarding the safety and sustainability of insect farming.

These research findings underscore the potential of insects as a nutritious and environmentally friendly alternative to traditional meat sources, offering both health and environmental benefits. Nevertheless, it is important to acknowledge that the utilization of insect protein as a substitute for conventional meat presents certain challenges and limitations, necessitating further research and development to unlock its full potential.

2.4. Plant-based proteins

Recently, plant-based protein sources have gained considerable attention as meat alternatives, mainly because of their health advantages and sustainability. People explore plant-based protein sources as potential meat substitutes. The plant-based food sector provides a diverse variety of protein-rich options, including soy products, seeds (such as almonds, chia, pumpkin), whole grains, legumes, nuts, lentils, chickpeas, tofu, tempeh, edamame, and even certain vegetables (Kumar et al., 2023). Besides providing several health benefits, plant-derived proteins generally have lower levels of saturated fatty acids and higher amounts of fiber, vitamins, and minerals. Research indicates that plant-based diets can reduce the risk of chronic illnesses such as heart disease, diabetes, and certain cancers (Wood and Tavan, 2022). Moreover, compared to meat, plant-derived protein sources offer greater environmental sustainability. Typically, plant-based protein sources require fewer resources and have a reduced impact on greenhouse gas emissions, and water pollution compared to livestock farming. In the subsequent sections, we present an overview of several plant protein sources commonly utilised as meat alternatives.

2.4.1. Soy protein isolates (SPI)

Soy protein, derived from soybeans, is renowned for its nutritional richness and complete amino acid profile, containing all essential amino acids (Thrane et al., 2017). Its nutritional composition and texture closely resemble those of meat, rendering it a low-saturated-fat and cholesterol-free alternative that is both versatile and sustainable, with notable health benefits. Furthermore, soy protein's versatility extends to its ability to mimic meat's texture when used in products like patties, sausages, or minced meat, offering a firm and chewy consistency akin to certain meats. Its adaptability can be further enhanced through flavouring and seasoning with various meat-like profiles (Singh et al., 2008).

The protein content of soybeans comprises about 35–40% with a well-balanced amino acid composition. In addition, soybeans contain approximately 15–20% fat, 30% carbohydrates, and 10–30% moisture. Soybeans are also a rich source of fibre, minerals (iron, calcium, and zinc), and B-group vitamins. Soy-protein products can be categorized based on their protein content to soy flour and grits containing 50% protein (dry basis), soy protein concentrate (SPC) with 70%, and SPI with 90%. Soy protein is a frequently employed meat analogue in research, enabling the creation of a variety of meat alternatives such as veggie burgers, meatless sausages, and meatless chicken nuggets. Moreover, it is crucial to consider the constituents used in texturing proteins, as they can either enhance or hinder the final product's quality. Components like starch, fibre, and microalgae have been explored for their impact on the density, colour, texture, and water absorption capacity of meat analogues.

The production of fibrous meat analogues employing oyster mushroom-soy protein through a single screw extrusion process has

been explored. Elevated oyster mushroom content resulted in a notable reduction in the expansion ratio of the extrudate. The moisture content in oyster mushroom extrudates surpassed that of protein mixture extrudates. Additionally, higher screw speeds and increased oyster mushroom content substantially augmented the water absorption index (Mohamad Mazlan et al., 2020).

Various structuring technologies have been investigated to produce meat alternatives obtained from plants. High-moisture extrusion (HME) is one of the most common and effective methods to produce plant-based meat alternatives with a fibrous structure and meat-like texture (Lee et al., 2023). Zhang et al. (2022) investigated the effect of different ratios of SPI and surimi on the gelling and textural properties of high-moisture extruded products (70%). The extrudate at SPI: surimi ratio of 80:20 resulted in the higher fibrous structure, chewiness, gel strength, and hardness, so that exceeding a surimi content of more than 40% hindered the fibrous texture. Anisotropic structure, which is fibrous textures resembling meat, can be created by plant protein processing through HME. Pietsch Bühler et al. (2019a) found out different process conditions such as extruder temperature (100–143 °C), extruder pressure (1.7–2.7 MPa), and specific mechanical energy input (85–350 kJ/kg) affected the formation of anisotropic structures in SPC. At these conditions, anisotropic structures occurred at high temperature and mechanical energy.

Extrusion cooking, a technology that can create meat-like texture and appearance from plant-based proteins have also been employed for production of soy protein-based meat. The impact of low-and HME cooking on physicochemical properties and structure of soy protein-based meat analogous alone or in combination with WG was investigated by Samard et al. (2019b). The result indicated that HME cooking with wheat gluten addition produced the most realistic and stable meat analogous, while LME cooking without addition of WG resulted in porous and expanded products.

Grahl et al. (2018) produced meat alternatives from soy and micro-algae spirulina (*Arthrospira platensis*) using HME cooking. The analysis of spirulina content and various technical factors in HME revealed that the concentration of spirulina (10–50%) and the moisture level employed in extrusion (57–77%) had the most significant influence on both the sensory characteristics and the measured texture attributes of the products. The temperature of processing zones in the extruder and the screw speed (600–1200 r/min) had a relatively minor impact on the final products attributes.

Vacuum packaging and pressurized heat (vacuum-autoclaving) treatment have been used by Woo Choi et al. (2023) as novel technology for producing high-moisture textured soy protein (TSP). The vacuum-autoclaving treatment decreased the texturization index and hardness of the TSP, indicating a decrease in overall structural strength and improved the textural characteristics of the TSP, making it more suitable for producing meat analogous. This treatment also induced protein aggregation and increased the disulfide bonds, β -sheets, and α -helices in the TSP, which contributed to structural changes (Woo Choi et al., 2023).

SPI also has been used as the main ingredient along with different types of hydrocolloids (such as xanthan, guar gum, and carrageenan) to improve the texture and stability of the meat analogues. Various methods (such as dynamic rheometer, texture profile analysis, and sensory evaluation) have been used to evaluate the effects of hydrocolloids on the viscoelasticity, hardness, springiness, cohesiveness and chewiness of the meat analogue (Nanta et al., 2021) such as, chicken salt-soluble proteins to increase water-holding capacity, mechanical properties and gelatin for improvement of the texture and stability of meat alternative (Lin et al., 2017).

However, there are some challenges using SPI as a plant-based protein. Food and Agriculture Organization of the United Nations (FAO) and the United States Department of Agriculture (USDA) included the soybean proteins as allergens. To address this issue, various conventional techniques like heat treatments, as well as innovative methods

involving pressure techniques (such as extrusion, high hydrostatic pressure, high-pressure homogenization, and controlled instantaneous pressure drop), and different waves-based treatments like gamma irradiation (γ -irradiation), pulsed ultraviolet light, cold plasma, microwave, and ultrasonication have been utilised to decrease the allergenicity of soybeans. Additionally, soy proteins contain naturally occurred ANFs such as trypsin inhibitors or those formed during alkaline/heat treatment such as D-amino acids and lysinoalanine, which can negatively affect human or animal health and reduce the protein and amino acid digestibility (Gilani et al., 2005). Therefore, there are several efforts for removal of ANFs from soy proteins. Haidar et al. (2018), tried to selectively remove ANFs such as trypsin inhibitors, isoflavones, and raffinose family of oligosaccharides from soy flour, while preserving its protein content and digestibility. They reported that aqueous micellar two-phase systems were a suitable tool to achieve this goal, as it allowed the recovery of 97% of isoflavones in the top phase, and more than 50% of the rest of ANFs in the bottom phase.

Consumer preferences for the flavors of soy-based food products are complex, with taste and texture emerging as key factors influencing their acceptance. A survey conducted by the United Soybean Board in August 2023 highlighted that taste ranks as the highest priority for consumers when considering plant-based foods and meat alternatives, followed by texture, health benefits, and affordability (Tachie et al., 2023). This emphasizes the importance of continuous innovation in soy protein ingredients to bridge the gap between taste expectations and actual product performance. However, the presence of a soy label on a product can negatively bias taste perceptions, leading consumers to perceive the taste as grainier and less flavorful. Conversely, when combined with a health claim, soy labeling can positively influence attitudes among health-conscious consumers, suggesting that the context in which soy is presented can significantly impact consumer acceptance. Thus, while taste remains a paramount concern, strategic labeling and marketing strategies can effectively navigate consumer preferences and drive the adoption of soy-based food products (Wansink et al., 2000).

2.4.2. Lupin proteins

Lupin, a leguminous plant, has witnessed a surge in popularity recently, primarily due to their notable nutritional value and potential health benefits. Lupin protein, derived from the seeds of the lupin plant, stands out for its complete amino acid profile, encompassing all nine essential amino acids. Its inherent low fat and carbohydrate content renders it an attractive choice for individuals focused on maintaining a healthy weight. Rich in minerals like iron, potassium, magnesium, and zinc, lupin protein serves as a valuable alternative to traditional protein sources like soy and whey, especially for those with dietary restrictions.

The impressive protein content and amino acid profile of lupin protein, combined with its water and fat-binding properties, have spurred extensive research into its potential as a meat alternative ingredient. Lupin protein exhibits a meaty texture when cooked, making it an ideal choice for meat alternatives. Studies have explored the physicochemical and sensory properties of beef sausages enriched with lupin flour (*Lupinus angustifolius*), revealing improvements in texture, adhesiveness, juiciness, and fat content upon the addition of lupin protein isolate (LPI) (Leonard et al., 2019). Similarly, lupin protein-derived meat analogues, when extruded with *Spirulina platensis* flour and under controlled parameters, demonstrated the potential to enhance meat analogues containing *Spirulina platensis* flour, thereby offering improved meat-like alternatives (Palanisamy et al., 2019). However, lupin protein may pose allergenic risks, and its availability worldwide is limited. Besides, the consumption of lupin is restricted due to the presence of various ANFs such as non-hydrolyzable oligosaccharides, phytic acid, alkaloids, and other secondary metabolites, which impair nutrient absorption and cause flatulence. Alkaloids are especially problematic due to their toxicity in mammals. Traditional methods to reduce these compounds, including soaking, boiling, dehulling, and washing, are laborious and not environmentally friendly. However,

biological methods using fermenting microorganisms have shown effectively reduced antinutritional factors and enhanced nutritional quality (Romero-Espinoza et al., 2020).

Lupin protein can be processed and prepared like beef or pork, rendering its viable substitute for traditional meat products. The nutritional characteristics of lupin-based meat analogues closely resemble those of meat. Additionally, lupin emerges as an eco-friendly and sustainable protein source, thriving in diverse climates and demanding fewer resources than conventional meat production. This makes it an appealing option for individuals seeking to reduce meat consumption or transition to plant-based diets. These studies collectively emphasize that lupin protein has versatile applications as a meat analogue source in a wide array of food products, backed by a growing body of research supporting its role in plant-based foods as a meat analogue.

Extraction and producing LPI from lupin seeds is a crucial step of investigating lupin as replacement of meat. Lupine protein concentrates (59%) were obtained by dry fractionation, which is a more sustainable method than wet extraction. Different milling and air classification conditions affected the protein content, yield, dispersibility, foam stability, viscosity, and digestibility of the lupine protein concentrates (Pelgrom et al., 2014). Abdullah and Abass (2019) obtained LPI with a high protein content (~90%) and a low-fat content (~2%) by extracting and purifying proteins from lupin seeds. The effect of different combinations of enzyme (alcalase, flavourzyme, and pepsin) were also investigated on the structural pattern, techno functional, and sensory properties of resulting peptides. The optimal enzyme combination was obtained at a combination of alcalase and flavourzyme at a ratio of 1:1 for producing hydrolyzed LPIs that can be used as a meat replacer (Abdullah and Abass, 2019).

Lactic fermentation is considered as a promising approach to reduce or mask the off-flavours of legum-based proteins. In this regard, fermentation of LPI polypeptides with seven different strains of *Lactobacillus* as well as *Staphylococcus xylosus* in a batch glass reactor under anaerobic condition for 24 h partially degraded the LPI polypeptides, especially those with low and medium molecular weight.

Evaluation of the sensory profile (aroma and taste attributes) of LPI and fermented LPI by a trained panel represented that fermentation reduced the pea-like and green bell pepper-like aroma of LPI and increased the popcorn-like and cheesy aroma (Schlegel et al., 2019). Sensory evaluation (colour, odor, taste, texture, and overall acceptability) of the burgers made with sweet lupine powder and camel meat by utilizing 20 untrained panelists using a 9-point hedonic scale shows that specific differences were observed in the sensory attributes of the burgers. According to the results, the sweet lupine powder burgers were rated significantly higher in terms of colour and odor, suggesting that these attributes were more appealing compared to the camel meat burgers. Conversely, the camel meat burgers received higher ratings for taste, indicating a preference for their flavor among the panelists, but not in terms of texture and overall acceptability (Abdullah and Abass, 2019).

2.4.3. Pea protein

Pea protein, derived from yellow split peas, has gained recognition as a versatile and valuable plant-based protein source. It is a commonly used component in various applications, including meat alternatives, meat analogues, sports nutrition products, and dietary supplements. Pea protein offers a more balanced amino acid profile compared to soy protein, making it nutritionally competitive. It is considered a complete protein, encompassing all essential amino acids the body requires but no longer synthesized endogenously. Additionally, pea protein is notably low in fat, carbohydrates, and calories, making it an ideal choice for individuals on weight management programs. Its ease of digestibility and hypoallergenic nature contribute to its widespread acceptance, as it rarely triggers allergic reactions (Lam et al., 2018). Notably, peas require less land and water for cultivation compared to other protein sources, rendering pea protein a sustainable and eco-conscious option.

Pea protein isolate (PPI) versatility and nutritional attributes make it a preferred ingredient in various applications, particularly in developing plant-based foods that imitate or substitute for meat. It is frequently employed to replicate the texture and taste of meat in plant-based food products [4]. The neutral flavour profile of pea protein makes it an excellent meat alternative, ensuring that it blends seamlessly with a wide range of food products without significantly altering their taste or texture (Liu, Cadwallader, and Drake). Consequently, pea protein finds extensive use in plant-based burgers, sausages, and meatballs, delivering a satisfying texture akin to traditional meat products (Giezenaar et al., 2024). As an alternative to meat, pea protein stands out for its excellent textural qualities and adaptability in food processing and cooking.

Low-moisture extrusion (LME) technology has been employed to process pea flour into textured pea protein with a fibrous structure and meat-like texture. Textured pea protein can be used as an ingredient in various plant-based food products that mimic the appearance, flavour, and nutrition of real meat. Examples are nuggets, patties, sausages, potstickers, crab cakes, and Mexican dishes. Textured pea protein has a clean flavour profile, a high protein content (59–79%), and no cholesterol. It also has a high hydration capacity (1.2–4.7 g water/g protein) and a short hydration time. It can be blended with textured wheat protein to improve the protein quality (Saget et al., 2021).

On the other side, high-moisture extrusion (HME) cooking, a process that can produce meat-like textures from plant proteins increased the solubility and hydrophobicity of PPI, indicating changes in the protein structure and exposure of hydrophobic groups. It also induced the formation of disulfide bonds and Maillard reaction products, which contributed to the cross-linking and browning of the PPI extrudates. Improving the water-holding capacity and oil-binding capacity of PPI, enhancing the textural and sensory attributes of PPI, such as hardness, chewiness, springiness, and juiciness, which are desirable properties for meat analogues are some of the other improvement caused by HME cooking (Osen et al., 2015). Different heat treatment conditions such as heating and cooling rates affect the structure, texture, and water-holding capacity of pea protein. According to X. D. Sun and Arntfield (2011), slow heating and cooling produced gels with higher water-holding capacity, lower hardness, and more porous structure than fast heating and cooling. Also, fast heating and cooling resulted in gels with higher hardness, lower water-holding capacity, and denser structure than slow heating and cooling. The gelation properties of pea protein were influenced by the formation of disulfide bonds and the denaturation and aggregation of protein molecules during heat treatment. Pea protein gels had similar or better water-holding capacity and texture than meat gels.

Pea protein enriched with some other ingredients such as lucerne, spinach and Chlorella was texturized using a single-screw extruder to improve the nutritional and sensory qualities of pea protein-based vegetal hamburgers (Peñaranda et al., 2023). Texturization improved the integrity and stability and reduced the hygroscopic features of the extruded materials. The presence of chlorophyll in the formulation increased the colour intensity during the cooking, so that the burgers containing Chlorella had the darkest colour and more similarity to the traditional meat hamburgers in terms of aroma and texture. Similarly, the blending of textured pea protein and textured wheat protein improved the protein digestibility, amino acid profile, and texture of meat alternative products (Maningat et al., 2022).

The flavor profile of pea protein is frequently characterized as neutral or mild, rendering it a versatile component in diverse culinary applications. In contrast to certain other plant-derived proteins which may possess pronounced or robust tastes, pea protein generally lacks overpowering flavor attributes. Its nuanced taste facilitates seamless integration with other constituents, exerting minimal influence on the overall gustatory profile of a product. This inherent neutrality renders pea protein a preferred selection for formulating meat substitutes and plant-based comestibles wherein the objective is to emulate the taste and mouthfeel of conventional meat products sans introducing competing flavor nuances (Shanthakumar et al., 2022). Moreover, the

subdued taste profile of pea protein lends itself to adaptation across a broad spectrum of flavor compositions and gastronomic methodologies. Concerning the flavor characteristics commonly associated with pea protein, it is widely acknowledged for its mild, cheesy, slightly saccharine, and earthy flavor profile. Such neutrality affords pea protein compatibility with an array of spices, herbs, and seasonings, thereby facilitating the development of flavourful plant-based meat substitutes that closely approximate the flavor of traditional meat products (Liu et al., 2023). Serving as a blank canvas for the infusion of additional flavor profiles such as herbs, spices, and seasonings, pea protein enables manufacturers to tailor the taste of their offerings in accordance with consumer preferences (Boukid et al., 2021).

2.4.4. Lentil protein

Lentils, diminutive, lens-shaped legumes, exhibit an array of colors, including green, brown, red, and black which are rich in protein, fibre, vitamins, minerals, carbohydrates, and bioactive compounds essential for human health.

Many researchers discussed the implications for using lentil protein as a value-added ingredient in food formulations. Different drying methods, such as freeze drying, spray drying, and vacuum drying have utilised to investigate the physicochemical and functional properties of LPI as a potential source of functional ingredients for food applications (Joshi et al., 2011).

Lentil protein has advantages over meat protein in terms of digestibility, sustainability, and cost (Barbana and Boye, 2013). However, different type of lentils has shown different chemical compositions which might be taken into account as meat alternatives. In this regard, Barbana and Boye (2013), prepared lentil protein concentrates by alkaline extraction and iso-electric precipitation from the lentil flours from Blaze and Laird varieties of lentil. Analyzing protein composition and properties showed that the Blaze variety had a lower colour difference and a higher hardness than the Laird variety. Fermenting lentil protein to improving protein quality and enhancing nonnutritive nutrients is one of the treatments to get the higher value and properties of lentil protein by changing in the secondary protein structure components, reducing the α -helix: β -sheet ratio, which was related to protein degradation. Water kefir seed were used to ferment lentil protein for 5 days by Alrosan et al. (2021). Fermentation led to an increase in lentil protein digestibility from 76.4 to 84.1%, indicating a higher bioavailability of amino acids, and in the total phenolic content from 443.4 to 792.6 mg of GAE/100 g, with chlorogenic and epicatechin as the predominant phenolic compounds.

When considering the sensory properties of lentil protein, it's essential to understand its multifaceted attributes that influence consumer perception. Lentils, characterized by their diminutive, lens-shaped form and a spectrum of colors ranging from green to black, bring not only nutritional value but also a distinctive sensory experience to food formulations. These sensory properties encompass aspects such as color, flavor, aroma, texture, and overall mouthfeel, all of which contribute to the palatability and acceptance of food products containing lentil protein (Shrestha et al., 2023). One of the primary sensory characteristics influenced by lentil protein is color. The varied hues exhibited by different lentil varieties, including green, brown, red, and black, can impart visual appeal to food products. Consumers often associate color with freshness, quality, and flavor intensity, making it a crucial factor in their sensory evaluation. Additionally, the color stability of lentil protein throughout processing and storage can impact the overall aesthetic appeal of food products, influencing consumer perception and purchase decisions (Lee et al., 2021; Zamuz et al., 2019). Flavor is another key sensory attribute influenced by lentil protein. Lentils possess a naturally earthy and nutty flavor profile, which can contribute depth and complexity to food formulations. However, the flavor of lentil protein can be further influenced by processing methods, such as fermentation or enzymatic treatments, which may enhance or modify its taste characteristics. Balancing the inherent flavors of lentil

protein with other ingredients in food formulations is essential to create harmonious flavor profiles that appeal to a wide range of consumer preferences (Shrestha et al., 2023). Texture plays a pivotal role in the sensory experience of food products containing lentil protein. Lentils exhibit varying degrees of firmness and mouthfeel depending on factors such as variety, processing techniques, and particle size. The texture of lentil protein can range from soft and creamy to firm and gritty, influencing the overall eating experience. Achieving the desired texture in food formulations often involves optimizing processing parameters, such as hydration levels, cooking times, and particle size reduction, to create products that are palatable and enjoyable to consume (Guo et al., 2024). Aroma is another sensory dimension influenced by lentil protein, albeit to a lesser extent compared to flavor and texture. While lentils themselves may not possess strong aromatic characteristics, their interaction with other ingredients and processing conditions can lead to the development of unique aroma profiles in food products. Roasting, toasting, or fermenting lentil protein can impart distinctive aroma notes, adding complexity and depth to the sensory profile of finished products (Usman et al., 2023).

2.4.5. Wheat gluten

WG boasts an exceptionally high protein content, typically ranging between 75 and 80% by weight, making it a pivotal ingredient for creating meat-like textures. WG forms a network of protein fibres upon hydration characterized by its viscoelastic and cohesiveness properties. This network can be manipulated to replicate the chewy and fibrous texture of meat, thus lending itself well to mimicking meat textures (Chiang et al., 2019).

Beyond its unique textural properties, WG assumes the role of a binding agent in the realm of meat analogues. By combining it with other plant-based ingredients, the desired texture and structure of meat analogues can be achieved. Consequently, WG stands out as an appealing component in the development of meat analogues, primarily due to its protein content and textural attributes. Nonetheless, the formulation of new products must consider various factors, including catering to individuals with allergies, enhancing flavour profiles, and accommodating diverse dietary preferences. Besides, the Maillard compounds, oxidized sulfur rich amino acids, and unnatural amino acids formed during the processing should be taken into consideration (Gilani et al., 2005). To create meat analogues that faithfully replicate both the flavour and texture of meat while addressing these considerations, WG is often employed in conjunction with other plant-based ingredients (Chiang et al., 2021).

Different process conditions including temperature, pressure and machine energy during HME processing affected the polymerization behavior of WG and the formation of anisotropic structures, which is the main factor determining the product quality (Pietsch et al., 2017, 2019b). WG polymerization increased with increasing barrel temperature, screw speed, and feed rate. The formation of anisotropic structures coincided with an increase in hardness and Young's modulus of the WG products. As conclusion, temperature was considered as most important parameter influencing WG polymerization. Higher temperature led to a higher degree of polymerization and more anisotropic product structure. While, pressure and machine condition had no significant influence on WG polymerization in the range investigated. WG polymerization only occurred in the screw section of the extruder, not in the die section. Twin-screw extrusion method has been used to produce WG/starch meat analogues in presence of different additives such as glycerol, water, and sodium stearoyl lactylate (Wang et al., 2017). The extruded products had higher hardness, lower cohesiveness, and darker colour than the raw materials, and that the additives affected the elasticity, springiness, and water-holding capacity of the products. Disulfide bonds formation, Maillard reaction products, and non-covalent reactions were responsible for WG/starch extrudate alternation.

2.4.6. Green leaf proteins

Green leaves from various crops have been studied as potential plant protein sources and valuable byproducts. These leaves contain a high concentration and well-balanced essential amino acid content, making them nutritionally beneficial. Various sources, such as alfalfa (20–25.75%), beet root (20%), cabbage (13.1%), cassava (11.8–38%) cauliflower (21.7%), Chaya (24.3%), rapeseed (12%), broccoli (12%), carrot (13.1%), and spent green tea leaves (21–31%), have been explored as protein sources for food ingredients (Heppner and Livney 2023). Adding green leaf proteins to foods can improve nutritional quality by providing essential amino acids and bioactive peptides from unconventional protein sources. However, human trials have indicated some negative effects due to ANFs such as saponins, tannins, mimosine, and tryptic inhibitors. ANFs such as phytic acid, trypsin inhibitors, and tannins in green leaf protein concentrates can be reduced using high-temperature processing (Biswas and Purohit, 2024). However, heat treatments may cause polyphenols to convert into tannins. Additionally, the toxicity level, particularly the cyanide content, is crucial for the safe use of green leaf protein. The method of extraction influences cyanide levels, with ultrafiltration producing lower cyanide content compared to acid thermo-coagulation (Biswas and Purohit, 2024). Protein digestibility in green leaf protein is higher than in their respective leaf meals due to lower levels of antinutritional factors and crude fiber. Although green leaf protein is rich in essential amino acids, they lack sulfur-containing amino acids and lysine, necessitating supplementation to enhance overall protein quality. The proteins in green leaves can be divided into a water-insoluble (green) fraction and a water-soluble (white) fraction. Rubisco, a white protein, has shown a good nutritional profile and is commonly found in green leaves (Perez-Vila et al., 2022). The extraction process for these proteins depends on factors like the source, species, harvest season, and region (Hadidi et al., 2023). Recent research by Pearce and Brunke (2023) has examined the potential of purified rubisco as a food ingredient, with some food companies expressing interest in using it as a meat replacement protein due to its cost-effectiveness, low environmental impact, and minimal processing requirements. However, more studies are needed to investigate the integration of extracted leaf proteins into food products. Understanding their molecular, physicochemical, functional, and biological properties is crucial for using these proteins effectively, particularly in meat analogues. Evaluating how these proteins behave under specific processing conditions and their interactions with other ingredients will determine their suitability as food ingredients, especially in meat replacement products (Heppner and Livney, 2023).

3. Key attributes of non-animal-based meat alternatives

Non-animal-based meat alternatives have witnessed a surge in popularity, primarily driven by concerns related to health, environmental sustainability, and animal welfare. However, for these alternatives to gain widespread acceptance, they must not only align with consumer expectations but also uphold environmental sustainability and nutritional value. Consumers typically demand specific product characteristics, encompassing physicochemical, textural, and sensory attributes, as prerequisites for their purchase decisions. Therefore, meat analogues must strive to closely resemble meat in terms of appearance, texture, aroma, and taste to effectively appeal to consumers with established meat consumption habits (De Angelis et al., 2020). Here are several key attributes that make plant-based meat alternatives highly desirable.

3.1. Physicochemical characteristics

It is imperative to underscore that the physicochemical characteristics of meat alternatives, encompassing water absorption capacity, porosity, oil absorption capacity, colour, and aroma, represent pivotal considerations within this context. The visual aspect of non-animal-

based meat alternatives should mirror that of conventional meat, typically exhibiting a reddish or brownish hue. Furthermore, the flavour profile of meat analogues should be characterized by savoury notes and an umami essence, while the aromatic profile should evoke the sensory attributes associated with traditional meat. A prevalent strategy employed in the formulation of analogues involves the inclusion of plant-based fats or oils to replicate the desired mouthfeel akin to that of meat. Various sources of fats are available to imitate the texture and mouthfeel of animal-based fat, including avocado and sesame oil or mixture of saturated and unsaturated fats like solid fats of coconut and canola oil with unsaturated sunflower oil. (Ishaq et al., 2022).

Numerous investigations have delved into the impact of plant proteins on the modification of physicochemical and functional attributes of meat analogues. For instance, a study by (Lee et al., 2022) juxtaposed SPI with rice protein isolate (RPI, 25–100% replacement) in the development of textured rice protein. Their findings revealed that the amalgamation of both protein isolates led to enhancements in water absorption capacity, porosity, and the specific mechanical energy of the resultant meat analogues compared to the commercial Hokyung textured soy protein R. In a parallel study, it was demonstrated that various plant-based meat analogues could be crafted from four different combinations of SPI, PPI, pea protein dry-fractionated isolates (PDF), and oat proteins (OP) including PDF-OP (70:30), PPI-OP (70:30), PDF-PPI-OP (35:35:30), and SPI-OP (70:30). These investigations showcased that pea protein extrudates exhibited a notable capacity for oil absorption to better formulation of fat in meat alternatives alongside a favourable sensory profile. Conversely, products formulated with PDF manifested lower hardness in comparison (De Angelis et al., 2020). Likewise, plant proteins have exhibited the capability to engender restructured products endowed with desirable texture profiles and fibrous structures. Notably, these products have shown similarities in integrity index, nitrogen solubility, cutting strength, and chewiness when juxtaposed with chicken samples (Chiang et al., 2019).

To further advance the development of enhanced non-animal-based meat analogues, it is imperative to undertake more comprehensive and in-depth investigations. Additionally, Chiang et al. (2021) delved into an examination of the physicochemical properties of meat analogues fabricated through the combination of WG and SPI, with a subsequent comparative analysis against commercially available steamed chicken. Their study demonstrated that a higher concentration of SPI contributed to the improvement of lysine content in the meat analogues from 1.40 to 2.58 mg/100 mg in meat analogues containing 0–60% SPI, although remaining notably lower than that of steamed chicken. Protein solubility analysis revealed that hydrogen bonds, disulfide bonds, and hydrophobic interactions assumed pivotal roles in the formation, stabilization, and preservation of structures in meat analogues.

In summary, the physicochemical properties of meat alternatives exert a significant influence on the overall quality and acceptance among consumers. The meticulous selection of ingredients, processing methodologies, and formulation strategies by meat alternative manufacturers is instrumental in optimizing these attributes. Alternative meat products hold the potential to provide consumers with a sustainable and ethically sound alternative to traditional meat products by attaining an appealing equilibrium of physicochemical characteristics. Ongoing research and innovation within this domain are poised to enhance the physicochemical attributes of meat alternatives further, rendering them increasingly appealing and accessible to a broader consumer base.

3.2. Textural and structural characteristics

Meat alternatives reproduce texture as a key characteristic, yet it is one of the most challenging to achieve. Numerous factors come into play, influencing the texture of meat analogues, including parameters like water content, protein structure, and the conditions employed during the cooking process. It is essential that a non-animal meat alternative mimics the chewy, tasty texture akin to traditional meat, as

texture significantly influences the success of these products as credible substitutes for real meat. As an increasing number of individuals opt for non-animal-based diets and seek sustainable food options, the ability to accurately mimic meat texture becomes ever more critical (Abbaspour et al., 2023). In order to achieve the desired texture, ingredients must be combined with exact processing techniques and continuous scientific innovation aimed at creating a product with a mouthfeel that replicates meat as closely as possible. Researchers are actively working on engineering meat analogues that closely mimic the texture and appearance of genuine meat. Researchers have explored various texturizing agents derived from natural origins in order to refine the texture of meat analogues. The ingredients in these agents include fibers, gums, and hydrocolloids, which work together to create a meat-like texture (Taghian Dinani et al., 2024).

Chiang et al. (2019), highlighted that when relying solely on wheat gluten (WG) for the development of meat analogues, there is a compromise in the chewy quality. However, the introduction of soy protein isolate (SPI) serves to enhance both chewiness and hardness. In a parallel study, Bakhsh et al. (2023), employed texturized SPI and texturized vegetable protein isolates (TVP) in conjunction with varying concentrations of methylcellulose (1.5, 3, and 4%) to fabricate meat analogues. The formulation resulted in acceptable textural profiles and crude fiber proportions. Yuliarti et al. (2021), conducted a study wherein they engineered a meat substitute using a combination of pea protein and wheat protein in different ratios of 17:0, 13:4, 8.5:8.5, 4:13, and 0:17. The meat substitute formulated with the ratio of 4:13 (pea protein: wheat protein) was the most preferred when contrasted with other combinations. The incorporation of pea protein in the analogues led to an augmentation in hardness, chewiness, and viscoelastic properties. Conversely, an increase in the proportion of wheat protein in the formulation resulted in a decrement in the textural and viscoelastic properties of the analogues.

Majzoobi et al. (2017), performed a study investigating the influence of specific hydrocolloids, including κ -carrageenan, konjac mannan, and xanthan gum (XG), on the sensory properties of meat-free sausages formulated from texturized soy protein, SPI, vegetable oil, corn starch, and spices. The findings highlighted that κ -carrageenan and konjac mannan positively impacted texture and overall acceptability, while XG showed more limited effects in these aspects. Among the hydrocolloids

studied, κ -carrageenan, followed by konjac mannan, exhibited the most significant efficacy in enhancing the meat-free sausage's overall quality. Various approaches can be used to improve the textural characteristics of meat analogues and plant-based meat alternatives. Using techniques such as adjusting extrusion profiles, utilizing high-pressure processing, and modifying screw speed can produce fibrous, meat-like textures. Moreover, optimizing formulations, adjusting pH levels, and using post-processing techniques like cooking, freezing, or thawing play important roles in improving plant-based meat texture. Ultimately, extensive research, development, and optimization efforts are essential to achieve the desired texture in plant-based meat analogues.

Achieving acceptance among traditional meat consumers necessitates that non-animal-based meat alternatives closely emulate the structural composition of real meat. The visual presentation of a product plays a crucial role in motivating consumers to consider dietary changes and reduce traditional meat products. While almost every non-animal protein holds potential for use in developing meat substitutes and alternative products, the unique globular structure of legume proteins poses challenges in mimicking meat's fibrous texture (Fig. 2). Achieving a structure and appearance similar to that of animal protein-based products requires the incorporation of a diverse range of functional ingredients. Creating meat-like fibrous structures involves complex processing techniques such as three-dimensional (3D) printing, thermos-extrusion, shear, spinning, and cross-linking. These methods alter the native protein structures, unfolding and denaturing them to facilitate interactions between proteins and carbohydrate polymers. Additionally, red pigments are introduced to enhance visual appeal, closely mimicking meat. The addition of various vitamins and minerals is also essential to ensure that the plant-based alternative has a nutritional profile comparable to meat products (Baune et al., 2022).

Researchers have adopted diverse strategies to enhance the structural attributes of plant-based protein meat analogues, with the objective of rendering them more closely akin to genuine meat. Certain investigations have explored the blending of various plant proteins to optimize their interplay, resulting in a more coherent and robust structural framework. In this context, proteins undergo a process of melting and denaturation. Temperature, shear, pressure, and moisture collaborate to ensure the complete dissolution of plant proteins during processing. Oxidation plays a pivotal role in exposing sulphhydryl groups

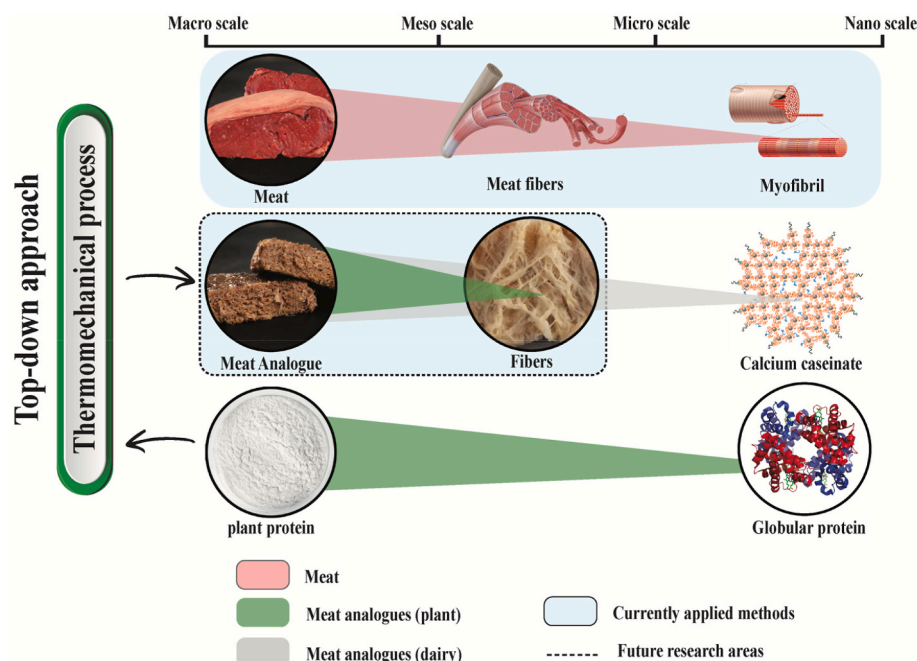


Fig. 2. Different texture and structure methods, their potential for meat and meat analogues, and potential future research areas (Schreuders et al., 2021).

within the protein chain, engendering new disulfide bonds. Consequently, the protein forfeits its original spherical structural integrity, adopting an anisotropic structure after denaturation. In this molten state, protein molecules are rearranged through interactions with other substances. Subsequent to homogenization and orientation, the mixture is subjected to cooling and characterization. This transformation of the protein into a tissue-like protein gives rise to a fibrous structure reminiscent of animal meat. To facilitate this process, the proteins employed as raw materials must inherently possess sufficient sulfur-containing amino acids within their globulin chains (Zhao et al., 2023).

Additionally, scientists have delved into the incorporation of gelling agents, such as hydrocolloids, to augment the binding and structuring of plant proteins, thereby enhancing the quality of meat analogues. Moreover, moisture exerts a considerable influence on the fibrous structure of tissue proteins. It orchestrates the unfolding and arrangement of protein molecules, contributing to the creation of fibrous structures. Previous research has underscored the significant impact of moisture content on fibrous structure formation during lupin protein extrusion. When the moisture content dropped below 40%, insufficient hydration of lupin protein occurred, resulting in an ineffective cross-linking process. Consequently, the resulting protein fibers exhibited inadequate structuring and were prone to breakage. In high-moisture processing, protein molecule aggregation is primarily driven by hydrophobic interactions. On the other hand, in low-moisture processing, disulfide bonds play a key role in stabilizing protein molecule aggregation, displacing hydrophobic interactions. In the range of 20–60% moisture content, disulfide bonding and hydrophobic interactions have a synergistic interaction, resulting in increased fibrillation. During processing, high levels of free moisture act as lubricants, which improve material fluidity, reduce processing pressure and intensity, reduce expansion occurrences, improve product structure compactness, and result in dense tissue protein structures (Guo et al., 2024).

In a separate study, various ratios of rice bran (RB) were incorporated into simulated meat using a high-moisture extrusion (HME) process with SPI to examine its structural properties. A microstructure analysis revealed a unique gel network structure in the RB-SPI simulated meat produced by HME. Simulated meat without RB exhibited a greater number of dense mesh structures at 30 \times magnification, whereas samples containing 15 and 20% RB showed a smaller number of dense mesh structures. When magnified to 50 \times , the internal structure morphology of simulated meat with 15 and 20% RB content was similar. However, it had fewer mesh structures than its RB-free counterpart. A comparison of mesh size in the gel network structure revealed that RB increased the gel network structure when magnified at 1500 \times (Jiang et al., 2022). Another study used pea protein isolate (PPI) to produce a low moisture meat analogue (LMMA) with mycelium (MY) ranging from 0 to 40% (w/w). According to structural analysis, the LMMA has the highest density when 100% PPI is used, while density decreases as MY increases. Increasing the MY content resulted in a more fibrous and permeable structure. Based on a microscopic examination, it was revealed that the aggregated network had a rigid structure. The formulation's morphology was linked to its water-holding capacity (WHC), with a porous structure leading to an increased WHC and, in turn, facilitating reconstituted fluids (Zhong et al., 2021).

The functionality of plant-based meat analogues compared to their meat counterparts is crucial, necessitating an exploration of the complexities involved in creating products that not only resemble but also function similarly to animal meat. The challenge resides in replicating the delicate balance of taste, texture, and nutritional profile characteristic of traditional meat products (Lee et al., 2020). Plant-based meat analogues strive to mimic the sensory experience of consuming meat, emphasizing aspects such as texture, juiciness, and flavor. Yet, the functionality of these products transcends mere imitation; they must also fulfill nutritional equivalence and satisfy the practical requirements of daily meals. This encompasses elements like cooking performance, shelf life, and preparation ease, which are vital for consumer acceptance

and adoption (Lee et al., 2020).

Plant-based meat analogue production employs advanced processing techniques aimed at altering protein structures and incorporating functional ingredients to boost the product's texture and nutritional profile. Techniques such as 3D printing, thermos-extrusion, and cross-linking enable the fabrication of fibrous structures that emulate the texture of meat. Likewise, the addition of red pigments, vitamins, and minerals ensures that the nutritional content of plant-based alternatives matches that of meat products (Sha and Xiong, 2020; Toh et al., 2024). The functionality of plant-based meat analogues is assessed based on their capability to withstand various cooking methods, including grilling, frying, and baking. This factor is essential as it determines whether these products can effortlessly fit into current meal plans and recipes, enhancing their attractiveness to consumers moving away from animal-based proteins (Chen et al., 2023a).

Overall, plant-based meat analogues aim to replicate the texture, appearance, and mouthfeel of animal-derived meat using plant-derived ingredients. The specific structure of a product depends on the ingredients, processing methods, and desired end product.

4. Nutritional aspects of meat alternatives

With changing dietary trends and increasing demand for sustainable protein sources, meat alternatives' nutritional attributes have come under increased scrutiny. This scrutiny is largely due to concerns about high processing levels. Many consumers and health experts worry that the processing involved in creating meat analogues might detract from their nutritional value and overall health benefits. Highly processed foods are often linked to negative health outcomes, such as higher sodium levels, preservatives, and additives. Moreover, the processing methods used might involve significant energy and resource consumption.

This section offers a comprehensive exploration of the nutritional compositions found within various plant-based proteins, which serve as integral components of meat analogues.

Plant-based proteins form the bedrock of meat alternatives, underpinning their nutritional constituents (Sha and Xiong, 2020). These proteins, including SPI, pea proteins, WG, lupin proteins, and others, comprise the fundamental building blocks of meat analogues (Zhang et al., 2021; Taghian Dinani et al., 2023; Qiu et al., 2023). Each of these protein sources exhibits a distinct nutritional footprint. For instance, SPI are renowned for their elevated protein content and amino acid composition closely resembling that of conventional meats (Cruz-Suárez et al., 2009). In contrast, pea proteins are celebrated for their remarkable digestibility and rich amino acid content, rendering them indispensable for the nutritional value of meat alternatives. This investigation into the nutritional characteristics is paramount in assessing the suitability of these proteins as substitutes for meat (Kaleda et al., 2021).

To truly assess the nutritional significance of meat alternatives, a comparative analysis with traditional meat sources becomes imperative. This segment rigorously examines the distinctions and similarities between plant-based proteins and meat, taking into account parameters like protein content, carbohydrate, fat content, and other critical nutrients (Table 2). This evaluation contributes to the establishment of their nutritional worth, reinforcing their position as proponents of health-conscious dietary choices. Table 2 shows that several meat alternatives, such as the Beyond Burger and V2 Burger, have similar protein amounts. The Beyond Burger includes 20g of protein per serving, equivalent to the Woolworths beef burger's 18g. Compared to typical meat, meat substitutes contain greater amounts of carbohydrates. For example, vEEF chicken nugget and Good Mix Burger contain 12.3g and 18g of carbohydrates per serving, respectively. The fat content of different alternatives made from plants varies significantly. The vEEF steak has much less fat (1.8g per serving) compared to the Market Value beef burger (22.2g). The Beyond Burger has 5 g of saturated fat, which

Table 3

Processing methods of meat analogous and their parameters and properties.

Plant-protein sources	Other ingredients	Processing methods	Main parameters	Properties	Reference
SPI	Wheat starch	HME	Moisture content: 60–70%; extrusion temperature: 138–160 °C and dry feed rate: 6.80 kg/h	A more layered and fibrous structure with solid toughness, chewiness, and high cohesive force	Lin et al. (2002)
Peanut protein powder and SPI	Wheat starch	HME	Moisture content: 55%; extrusion temperature: 110 °C; and dry feed rate: 6.00 kg/h	A dense fibrous structure, low hardness, and a high springiness	Zhang et al. (2020b)
SPI and gelatin		Electrospinning	Acceleration voltage: 25 kV; nozzle-collector distance: 7.5–15 cm; nozzle diameter: 0.61 mm and flow: 3–20 $\mu\text{L}/\text{min}$	A more stable fibre structure and improved spinnability	Nieuwland et al. (2014a)
SPI and zein		Electrospinning	Electrostatic voltage: 25 kV; and nozzle collector distance: 12–14 cm	Optimum performance for spinning	Phiriyawirut et al. (2008)
SPI and WG	Corn starch	HME	Moisture content: 70%, extrusion temperature: 130 °C	A higher integrity index, higher springiness stability, and a greater cutting strength	Samard et al. (2019b)
SPC and WG	Wheat starch and pumpkin power	HME	Moisture content: 60%; extrusion temperature: 170 °C and dry feed rate: 2.8 kg/h;	Texturized, fibrous, hard and chewy product	Chiang et al. (2019)
SPI	Maltodextrin	Electrospinning	Experimental temperature: 21–25 °C; and relative humidity: 10–15% and Nozzle-collector distance: 15.5 cm	Increased spinnability and decreased viscosity	Kutzli et al. (2019)
Microalgae and soy protein		HME cooking	Screw speed: 100–300 rpm, die diameters: 3 and 5 mm,	Lower moisture content led to a more tender and less chewy product	Caporgno et al. (2020)
SPI, Mung bean protein isolated, peanut protein isolated, PPI, and WG		Moisture extrusion	Feed rate: 100 g/min, feed moisture: 50%, screw speed: 250 rpm., and barrel temperatures: 100, 160, and 140 °C from feeding to die zones	WG-based TVPs: High textural properties, low integrity index, and low essential amino acids content. IPP-based TVP: highest quality of meat analogue with high rehydration, textural, and emulsion properties as well as a high level of essential amino acids	Samard and Ryu (2019b)
PDF, PPI, SPI, and OP		LME cooking	Moisture content: 15, 20, and 25%. screw speed: 100, 150, and 200 rpm. temperature profile: 100, 120, and 140 °C	PDF-OP and PPI-OP mixtures had the highest scores for colour, odor, taste, texture, hardness, cohesiveness, springiness, chewiness, and resilience as well as water and oil absorption capacity, and rehydration ratio	De Angelis et al. (2020)
SPI, WG, PPI, and RPI	Water, vegetable oil, salt, sugar, flavour, enhancer, colorant, and texturizer	HME cooking	Moisture content: 15–35%, barrel temperature: 120–180 °C, and screw speed: 100–300 rpm	Fibrous and porous structure. high acceptability in terms of odor, taste, and texture.	Samard and Ryu (2019a)
SPI and WG	Corn starch	LME and HME cooking	Feed moisture: 30% (LME) and 70% (HME), screw speed: 150 and 200 rpm, and temperature: 100, 160, and 130 °C	Nitrogen solubility index (NSI), integrity index, springiness, hardness and cutting strength	Samard et al. (2019a)
SPI, and OP	Water	LME cooking	Screw speed: (225–800 rpm, temperature profile: 100–140 °C, moisture content: 20–35%, and specific mechanical energy: 0.5–1.8 kJ kg^{-1}	Fibrous texture, cylindrical shape, high protein content, low lipid content. good water and oil absorption capacity	De Angelis et al. (2020)
WG	Water	HME	Barrel temperature: 100, 125, 155 °C, screw speed: 180, 400, 800 rpm, feed rate: 10, 20 kg/h	The hardness and Young's modulus increased with increasing barrel temperature and screw speed, while the SDS-extractable protein decreased	Pietsch et al. (2019b)
Pea protein and wheat protein		Freeze structuring technique	–	Pea protein: increased the hardness, chewiness, and viscoelasticity of the analogue. Wheat protein: decreased hardness, chewiness, and viscoelasticity of the analogue	[11]

continuous and discontinuous phases.

HME offers various advantages, including improved efficiency, reduced waste discharges, higher energy efficiency (~ 200 – 1200 kJ/kg), and the production of more texturized products. However, high-moisture extruded meat substitutes may have limitations in terms of juiciness and texture, which can potentially be enhanced through a redesigned HME process founded on a fundamental understanding of structure-function relationships.

Specific plant proteins have been recognized for their role in facilitating the development of fibrous structures through extrusion. Lin, Huff, and Hsieh ([Lin et al., 2002](#)) conducted research using wheat starch and SPI extruded at varying temperatures (138, 149, and 160 °C) with moisture contents of 60, 65, and 70%, respectively. The findings

indicated that product sensory attributes and extrusion process parameters were more influenced by moisture content than cooking temperature. Lower moisture content resulted in higher die pressure and product temperature, yielding products with greater rigidity, chewiness, cohesiveness, and more layered and fibrous structures. Increased extrusion moisture and cooking temperature were associated with enhanced water absorption capacity. A study conducted by [Osen et al. \(2014\)](#), compared three different commercial PPIs to investigate how protein properties influenced the response of the extruder and the texture of the resulting products. The findings revealed that, despite their similar chemical compositions, the functional properties of PPIs had a significant impact on mass protein viscosity during the initial heating phase of the extrusion process. While there were notable

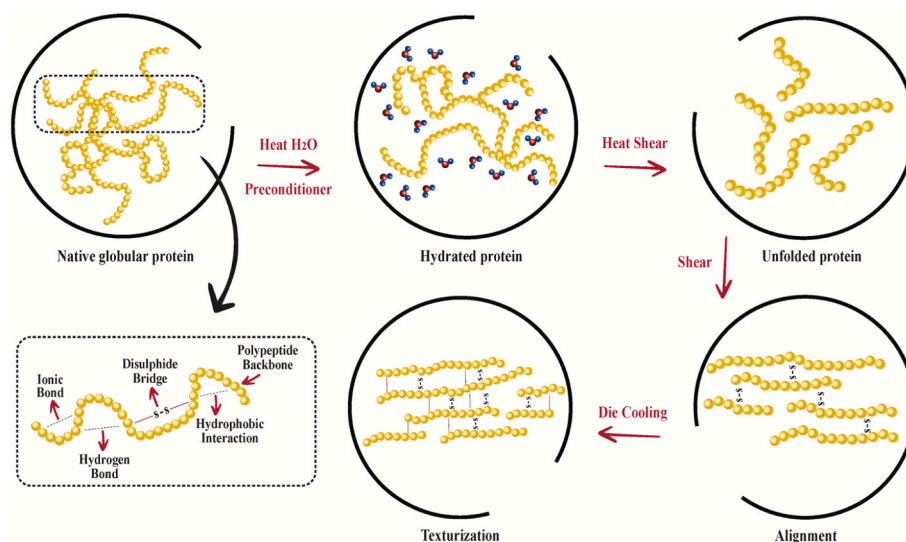


Fig. 3. Protein structural changes during extrusion cooking (Vatansever et al., 2020).

differences in the energy input required for texturization, product texture properties were predominantly influenced by cooking temperature and exhibited similarity across the various proteins. This research highlighted that PPIs are a highly effective raw material for producing

fibrous whole-muscle meat alternatives, offering extensive possibilities for product development.

Additionally, Zhang et al. (2020a) reported that the extrusion temperature had a profound effect on the tensile properties and springiness

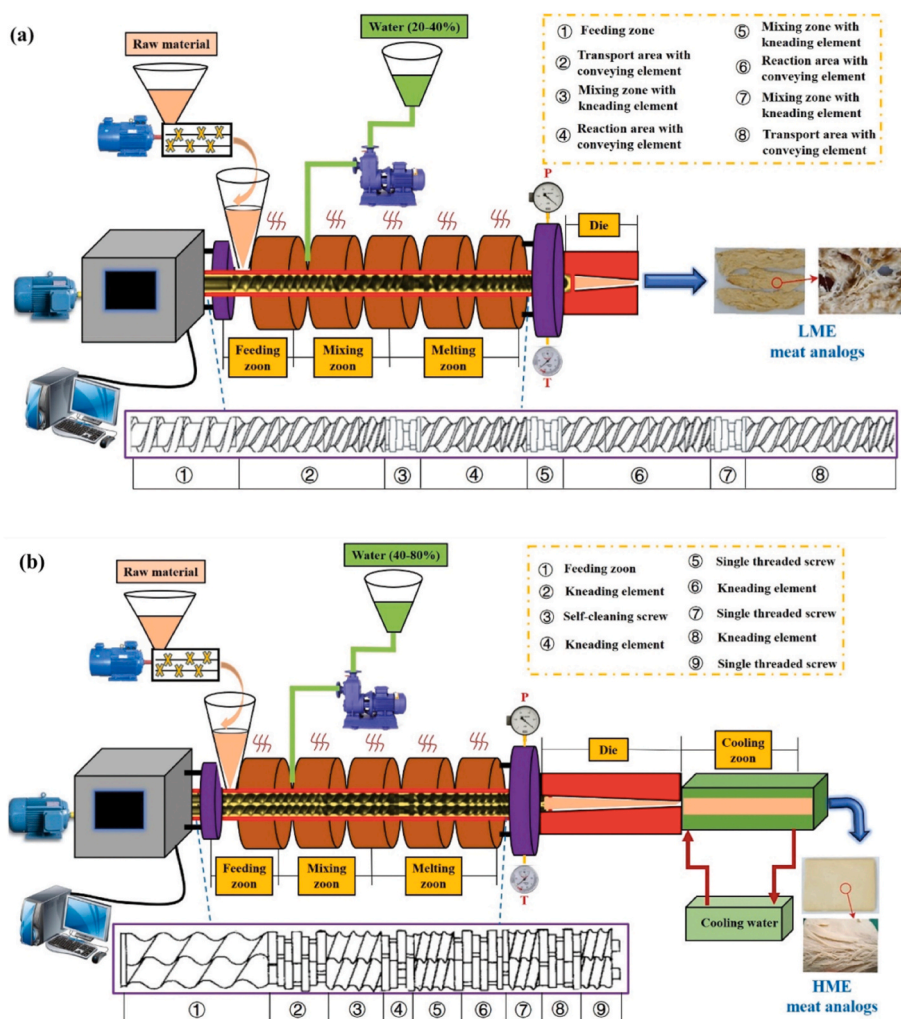


Fig. 4. Schematic representation of extrusion process equipment for (a) low-moisture extrusion and (b) high-moisture extrusion (Zhang et al., 2023).

of peanut protein, while moisture content had a more substantial impact on colour values and hardness. The results indicated a strong correlation between the characteristics of high moisture texturized peanut protein and the method of energy input. Fig. 4 illustrates a schematic representation of the extrusion process equipment used for both LME (Fig. 4a) and HME (Fig. 4b).

5.2. Protein spinning method

There are two primary methods of spinning technology, including electrospinning and wet spinning. Wet spinning involves the extrusion of a protein solution through a spinneret to create thin filaments within an insoluble protein solution, leading to the precipitation of proteins and fibre formation due to the interaction between insoluble and soluble solutions. Plant-based proteins from sources like soybeans and peas are suitable for wet spinning, but this method tends to have low production efficiency and can result in the generation of significant chemical waste when production demands are high (Nagamine et al., 2023). Electrospinning has become a preferred method over wet spinning. Electrospinning devices typically consist of injection push mechanisms, injectors, electrospinning nozzles, power supplies, motion platforms, and collectors. To perform electrospinning with a mixed plant protein solution, a syringe is filled with the solution, and then, using an injection push mechanism, the solution is extruded through an electrospinning nozzle. The high-voltage electric field generated between the collector and the electrospinning nozzle causes the solution to form a jet and deposit the fibres onto the collector. This technique enables the creation of nanoscale protein fibres using an electric field without generating chemical waste. Therefore, this process is more environmentally friendly and efficient compared to wet spinning (Yang et al., 2023; Imran and Liyan, 2023). However, it relies on the high solubility of fibrous or coiled proteins in a solution or molten state. Additionally, it cannot be applied directly to globular plant proteins like those found in soybeans and peas. Instead, proteins such as gelatin are used as carriers for electrospinning (Nieuwland et al., 2014a).

Electrospun fibres have diameters within the micro-to-nanoscale range, offering a high surface-to-mass ratio and allowing for precise control over their functional properties. Additionally, electrospinning enables the production of glycosylated proteins, which are more efficient and effective than traditional dry or wet methods. This enhanced efficiency is achieved by closely contacting the reactants inside the fibers during the stretching and bending process involved in electrospinning (Chen et al., 2023b).

To ensure a successful electrospinning process, specific criteria must be met, including the appropriate range of surface tension, electrical conductivity, and rheological properties of the polymer solution. Additionally, the concentration of the polymer must be sufficiently high to ensure the overlapping of molecules and the formation of an entangled network that resists stretching and bending during electrospinning. Several studies have effectively electrospun protein-polysaccharide mixtures, such as amaranth protein-pullulan, pea protein-pullulan, and whey protein-dextran (Aguilar-Vázquez et al., 2018; Kutzli et al., 2019). In all of these cases, using high molecular weight polysaccharides with long chains was necessary to ensure adequate entanglement in the spinning dispersions. Kutzli et al. (2019), successfully produced food-grade electrospun fibers using maltodextrin blended with whey protein isolate (WPI), SPI, and soluble SPI. The study revealed that the type of protein and its concentration in the spinning dispersion had a direct impact on the fibre production rate. Higher protein content generally led to increased electrical conductivity and viscosity. The study's findings indicated that SPI, in comparison to WPI samples, reduced the surface tension of the spinning dispersions and enhanced electrical conductivity and apparent viscosity. Furthermore, when only soluble SPI was utilised alongside maltodextrin for electrospinning, the viscosity decreased, resulting in improved spinning outcomes. Nieuwland et al. (2014b) explored the potential of creating fine fibrils to serve

as foundational components for visually appealing meat alternatives with desirable textures. The study employed gelatin as a carrier polymer for the electrospinning of globular proteins. This research marked the first food-grade application of electrospinning for producing composite fibres comprising globular proteins. The process of manufacturing protein composite fibres using both electrospinning is illustrated in Fig. 5.

In conclusion, electrospinning is affected by a multitude of factors, encompassing polymer characteristics like structure, concentration, and molecular weight, as well as solvent properties, including electrical conductivity, viscosity, and surface tension. Additionally, process-related aspects and environmental factors like temperature and relative humidity play pivotal roles in this technique. The primary challenge with electrospinning lies in finding polymers that possess both high concentration and solubility. It is imperative that proteins exhibit a random coil structure instead of their natural globular form, which can lead to insoluble aggregates upon denaturation. Some animal proteins, such as whey, collagen, egg, and gelatine, have already been successfully transformed into fibres through electrospinning. In contrast, plant proteins, which are typically globular, have primarily been used in the form of zein due to their random coil protein structure at the nanofibre level. However, a blend of certain plant proteins with suitable carriers like gelatin or other spinnable polymers like maltodextrin can also be electrospun into fibres, owing to the covalent attachment of carbonyl groups in maltodextrin to amino groups.

5.3. Freeze-structuring approaches

The freeze-structuring method, which involves freezing a protein emulsion to create a unique fibrous structure, has emerged as a promising technique for crafting fibrous non-animal proteins (Dekkers et al., 2018).

The freeze-structuring technique entails blending proteins with other components until a homogeneous emulsion is formed (Fig. 6a). Subsequently, the mixture is shaped, frozen, and allowed to undergo drying. Protein's fibrous texture is set at high temperatures without melting the ice crystals. Modifying freezing conditions, such as the freezing rate, pH, material solids content, surface properties, heat exchange characteristics, confinement degree, and pressure effects, can influence the textural attributes of the protein (Halonen et al., 2020).

The freeze-structuring technique holds promise for creating a fibrous and layered structure in plant-based nuggets, especially in situations where extrusion cooking is not available. Yuliarti et al. (2021) employed the freeze-structuring method to produce plant-based nuggets using different ratio of the pea protein to wheat protein (17:0; 13:4; 8.5:8.5; 4:13, and 0:17). A higher ratio of wheat protein (4:13) proved to be the most favoured analogues compared to controls. These particular

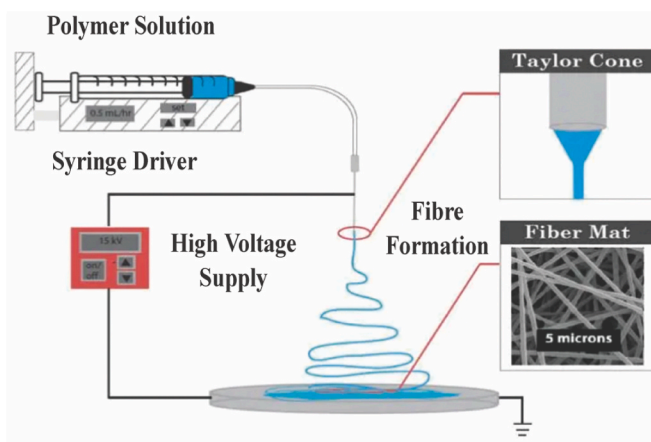


Fig. 5. Manufacturing plant proteins composite fibres with electrospinning (Wang et al., 2023b).

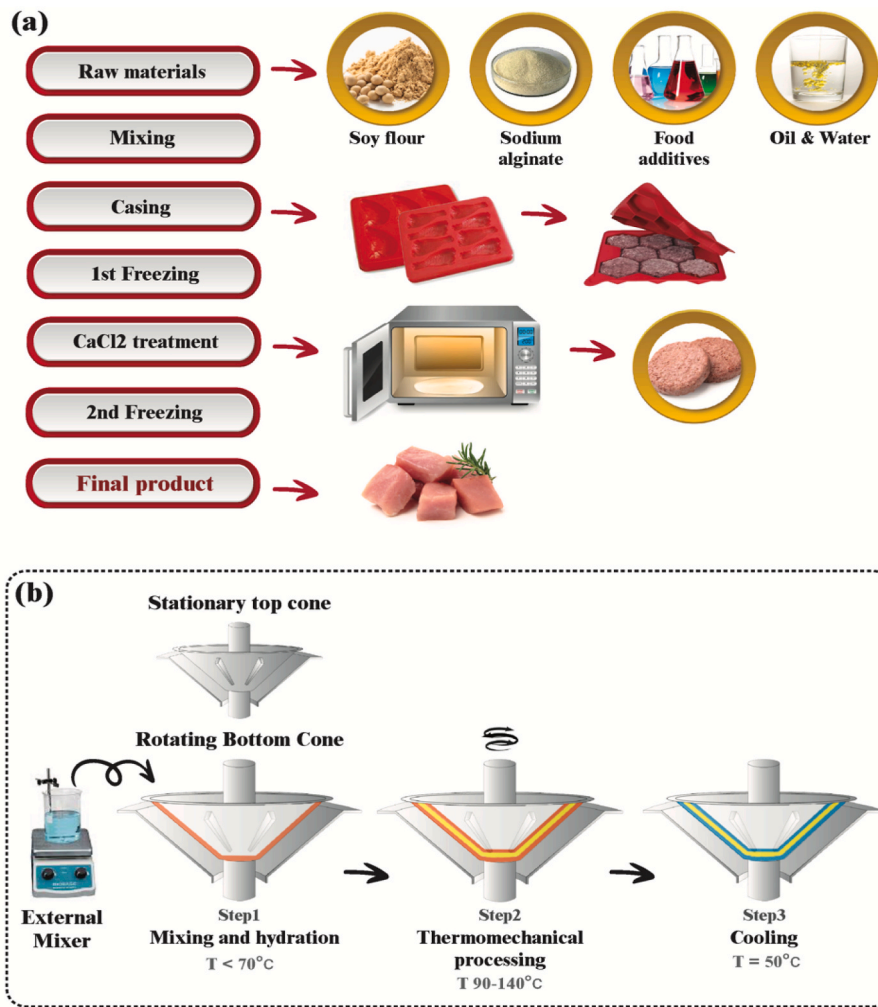


Fig. 6. (a) Schematic illustration of the freezing method and (b) shell cell process (Chantanuson et al., 2022; Baune et al., 2022).

analogues exhibited fibrous and layered microstructures, with viscoelastic properties, which were significantly influenced by the degree of protein cross-linking. Additionally, higher levels of pea protein contributed to increased product hardness, chewiness, and viscoelasticity. In a similar study, the freeze-structuring method was applied to produce soy protein-based food gels. The investigation revealed that a formulation made from soybean flour with a 10% solid content yielded a dense, two-layered, and porous structure with a mechanical strength similar to reference meat. However, freezing was found to be ineffective for texturizing formulations with high solid content or soy protein isolate (SPI) that had high gelling capabilities. Consequently, it was essential to mitigate the soy protein's gelling ability to achieve an anisotropic, meat-like structure through the freezing technique. Additionally, the resulting structures were affected by freezing rate and duration, requiring optimization for scalability purposes (Chantanuson et al., 2022).

In general, the freeze-structuring technique can create fibrous structures that resemble muscle fibres in meat, enhancing the overall sensory experience. However, this process involves several steps and can be time-consuming, which may limit its scalability in the production process.

5.4. Shear cell technology

Non-animal proteins can be manipulated to simulate the texture and mouthfeel of meat using shear cell technology, which offers an effective

approach for producing meat substitutes. The shear cell process relies on flow induction technology, which involves blending plant-based proteins with water and other components like starch, fibre, and flavourings (Manski et al., 2008). In shear cell technology, two similarly shaped plates, preheated, are set in motion to shear the proteins. The process entails preheating a shear pool, followed by the addition of the pre-mixed ingredients to heat and shear the plant proteins. The reaction that takes place in the shear tank is more controlled, consistent, and thorough in terms of deformation compared to extrusion technology (Fig. 6b).

Shear cell technology offers a diverse range of textures, including chewy and fibrous, as well as tender and juicy meat analogues. This versatility allows non-animal products to effectively compete with traditional meat products in terms of flavour, texture, and nutritional value. Additionally, shear cell technology enables the production of significant quantities of meat analogues in a scalable and cost-effective manner. Furthermore, the constant shear force applied during non-animal-based meat processing by shear cell technology results in approximately 10% lower mechanical energy consumption compared to the extrusion process (Krintiras et al., 2015). This method also allows for the use of relatively mild processing conditions, such as 95 °C at 30 rpm for 15 min at the laboratory scale or 120 °C at 20 rpm for 30 min at the pilot scale (Baune et al., 2022).

Jia et al. (2021), discovered that the development of fibrous materials in shear cells is more favourable when plant-based ingredients possess two distinct phases that undergo deformation and alignment

during shearing. This can be achieved by blending pure components with varying water-holding capacities, such as SPI and WG. Alternatively, such phases can be found in less purified natural ingredients, like SPC. In this regard, the structural potential of rapeseed protein concentrate (RPC) in the presence and absence of WG was investigated for the production of meat analogues using shear cell technology. The findings revealed that at 140 and 150 °C, with a dry matter content of 40%, both RPC-only and RPC-WG mixtures exhibited fibrous structures. Furthermore, the addition of WG enhanced the fibrous structure and lightened the colour. The shear-cell method offers the advantage of producing more extensive and thicker meat-like products, not limited to thin strands, thereby resembling whole muscle parts from animals.

5.5. Three-dimensional (3D) printing technology

Recently there has been a notable paradigm shift within the meat analogues industry, marked by the assimilation of advanced 3D printing technology. Commonly referred to as additive manufacturing, 3D printing heralds a pioneering method to craft meat alternatives marked by extraordinary precision. This disruptive technology offers meticulous control over the composition and structure of plant-based proteins, pursuing the emulation of the texture, visual attributes, and even gustatory aspects inherent in conventional meat products (Qiu et al., 2023). The fundamental premise of 3D printing hinges upon a layer-by-layer assembly approach, which involves the sequential deposition of materials, generally in a paste or gel-like state, to fashion a three-dimensional construct methodically. Each layer is intricately designed, delivering an unprecedented level of customization in the creation of meat analogues (Shahbazi et al., 2022). In practical application, plant-based constituents are transmuted into a printable amalgam, including diverse elements such as protein substrates, binding agents, flavour enhancers, and other essential components. The formulation of this composite is of paramount importance, as it substantiates the pursuit of achieving the desired texture and sensorial characteristics in the ultimate product (Singh and Sit, 2022). A remarkable facet of 3D printing in meat analogues is the inherent degree of individualization and customization it offers. Individuals have the liberty to specify particular parameters for their meat substitutes, encompassing the choice of protein origin, flavouring components, and even the visual presentation of the eventual product. This extensive scope of customization aligns harmoniously with the burgeoning trend of tailoring food to cater to individualized dietary requisites, preferences, and nutritional prerequisites (Wang et al., 2022). It is noteworthy that 3D printing technology empowers manufacturers with an intricate command over the texture and structural attributes of meat analogues. This innovation facilitates the replication of nuanced internal structures, including marbling and fibrous textures, features typically elusive using conventional manufacturing methods (Qiu et al., 2023). Beyond the realms of personalization and textural regulation, 3D printing emerges as a paragon of resource efficiency and a harbinger of minimal waste generation. The capacity to craft intricate geometries with laser-like precision results in the judicious use of materials and a reduced ecological footprint, in stark contrast to established manufacturing techniques (Hai Alami et al., 2023). However, it is imperative to acknowledge that the application of 3D printing in the meat analogues domain is not devoid of challenges. The research community and industry stakeholders are actively engaged in addressing aspects related to optimizing printability, refining texture and flavour attributes, and enhancing the overall quality of 3D-printed meat analogues (Wen et al., 2022a, 2022b). Furthermore, the acceptance of this novel technique hinges upon consumer preferences, as the unconventional approach and customization options may be enticing to certain segments of the market but necessitate alignment with consumer taste expectations (Chen et al., 2022c). Peering ahead, the assimilation of 3D printing technology into the meat analogues sector is poised for expansive growth as continued research and development endeavours strive to hone this pioneering approach. As the technology matures, its

potential to revolutionize the landscape of plant-based meat product manufacturing becomes increasingly evident. The integration of 3D printing into the realm of meat analogues epitomizes the continuous pursuit of sustainable, customizable, and sensorial enticing non-animal-based alternatives to meat, thus exemplifying the convergence of technology, culinary innovation, and the evolving gustatory predilections of the modern food industry.

Qiu et al. (2023) evaluated the rheological properties and the performance of edible inks derived from SPI, WG, and rice protein in the production of 3D-printed meat analogues. The findings revealed that the protein-enriched inks exhibited pseudoplastic behaviour, accompanied by viscoelastic properties. Notably, as the proportion of rice protein increased in the formulations, there was a consistent decrease observed in the apparent viscosity and storage modulus of the pastes. This reduction in rheological parameters corresponded to improved 3D printing performance, as evidenced by enhancements in hardness, support force, and plasticization of the printed meat analogues. In another study, [37] aimed to closely replicate the red and brown colour attributes of meat, both in its raw and cooked states. For this purpose, 3D printable colorant-containing meat analogues formulated with mung bean-based protein in presence of xylose. The remarkable transformation in colour was achieved by capitalizing on the thermal lability of beet red and the Maillard reaction, demonstrating the versatility of such a formulation. The incorporating xylose elevated shear modulus, dimensional stability, and hardness. On the other hand, in the cooked state, this modification led to heightened hardness, accompanied by reductions in yellowness, lightness, chroma, and hue angle. The incorporation of xylose also manifested alterations in the interactions and microstructure of the colorant-containing meat analogues, which, in turn, translated into variations in texture. Demircan et al. (2023) formulated meat analogues for 3D printing using various protein sources (pea protein, soy protein, and wheat protein) fortified with different mushroom cultivars (reishi, saffron milk cap, and oyster). 3D printing performance was evaluated by variables such as nozzle height, printing speed, and flow compensation. The study identified that nozzle height and printing speed significantly influenced print accuracy and layer smoothness. All ink formulations exhibited suitable rheological properties for 3D printing, except for the lumpy appearance of the reishi variant. The saffron milk cap and oyster inks demonstrated re-printability, contributing to sustainability and waste reduction. Mushroom fortification improved juiciness, nutritional value, and the release of umami amino acids in printed meat analogues. Fig. 7 provides a schematic illustration of the production process for meat alternatives using 3D printing technology.

6. Current commercial applications and market trends

The popularity of non-animal-based meat analogues in the food industry has been on the rise, with several commercial companies offering these products (Table 4). Selecting the right non-animal-based ingredients to replicate the sensory qualities of meat closely can be a significant challenge for these companies. As a result, sourcing alternative proteins has been a key activity in this field (Chen et al., 2022b).

Soy protein is a widely used ingredient for meat analogues in the market, primarily due to its affordability and well-understood processing properties. Quorn products, on the other hand, utilize mycoproteins, which are textural proteins derived from fungi. Pea protein has gained popularity in HME meat analogues due to its promising properties related to emulsification, foam stabilization, and gel formation. Additionally, wheat protein is commonly employed for its excellent rheological and viscoelastic properties. Wheat protein, thanks to the presence of disulfide bonds in WG, can provide a uniform texture and a mouthfeel similar to that of real meat analogues (Shaghaghian et al., 2022). Plant-based protein sources are used to make a variety of products, including burgers, sausages, nuggets, meatballs, and more. However, they must also match the nutritional characteristics of real meat products, including amino acid profiles, digestibility, and

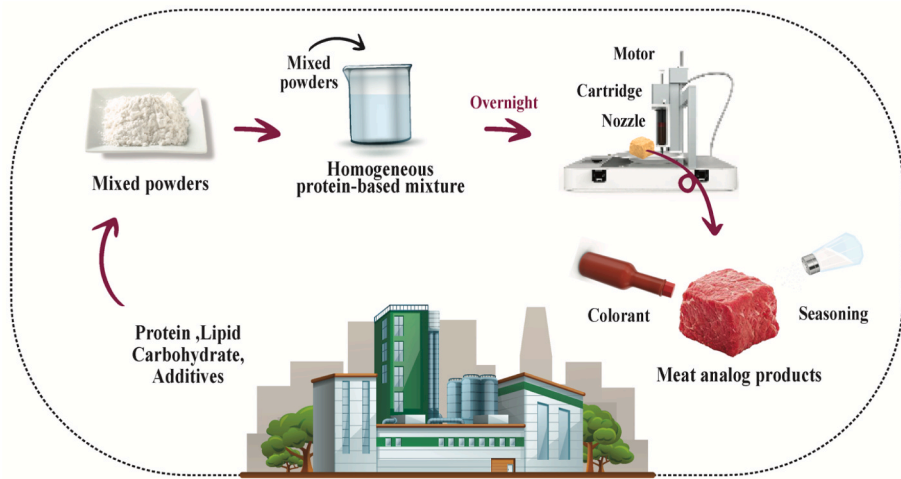


Fig. 7. Schematic illustration of meat alternatives production using 3D printing technology.

Table 4
Some examples of commercial meat analogues companies and their products.

Country	Company	Plant protein used	Plant-based products
USA	Impossible Foods	SPC, SPI, potato protein	Burger patties, ground meat, and sausage
USA	Morning Star Farms	Soy flour, egg whites, SPI	Veggie burgers, chicken nuggets, and breakfast items
Europe and North America	Quorn	Mycoprotein	Meatless nuggets, burgers, and sausages
Canada	Gardein	SPC, WG, SPI	Chicken style tenders, Beefless ground, and fishless filets
USA	Sweet Earth	Wheat protein, jackfruit, and legumes	Burgers, veggie burritos, deli slices, breakfast sandwiches
USA	Beyond Meat	Pea protein, rice protein, mung bean protein, faba bean protein, WG	Burgers, sausages, nuggets, chicken style fillet, meatball, mince, and smash
Hong Kong	Green Common	SPC, SPI, pea, and rice protein	Burgers, sandwiches, salads
USA	Rebellyous	SPC, SPI, and wheat protein	Nuggets
Australia	vEEF	Soy, pea, and wheat protein	Chicken style nugget, schnitzel, tender, roast, and burger, bacon style bits, beef style pieces, burger and steak
Australia	V2 Foods	Soy protein	Mince, sausages, schnitzel, burger

bioavailability, to avoid any unwanted health effects. The development of meat analogues by companies faces challenges, particularly in achieving the quality of organoleptic components, especially in terms of texture and taste, which are often considered inferior to real meat. Additionally, competitive pricing is essential for meat analogue acceptance. Accordingly, researchers need to prioritize the development of innovative protein foods and meat substitutes that closely mimic the characteristics of meat (Kumar et al., 2017).

7. Comparison of environmental impacts of plant proteins and animal proteins

There is a higher environmental impact associated with protein-rich foods than carbohydrates-rich foods. The global warming potential (GWP) of a protein source product is considerably depends on various factors such as product type, protein content, and geographical location

(Dindaroglu et al., 2023). Various studies on life cycle assessments indicated that meat alternatives generally have a lower environmental impact and use fewer resources than traditional meat. Research by Sagnet et al. (2021), found that production and consumption of cooked pea protein balls had a lower environmental burden across all 16 categories assessed compared to the beef meatballs. Similarly, Saerens et al. (2021) showed that plant-based burger patties (soymeal and pumpkin seed flour) had a lower environmental impact (>10 times) than meat burger patties (beef, pork, and chicken) per mass. In both studies, meat products exhibited significantly higher impacts on acidification (>5 times), ecotoxicity (>4 times), land use (>3 times), and photochemical oxidant formation (>2 times) compared to meat alternatives. In addition, they showed that meat products have higher ozone depletion (>40%) and beef has higher freshwater eutrophication (>3 times) than meat substitutes. In another study, a life cycle assessment of a plant-based burger patty compared to a beef burger showed its lower environmental impacts, including a 65% reduction in global warming potential and a 45% reduction in water consumption. This study suggested that switching from beef to plant-based patties in the UK population could save 3 million tonnes of CO₂e annually, which is equivalent to 0.74% of the country's yearly territorial greenhouse gas emissions (Tang et al., 2024). It is important to note, however, that plant proteins' environmental effects vary depending on their sources and processing methods (Santo et al., 2020). Some plant-based proteins require various chemical and mechanical treatments to enhance their nutritional value and texture, resulting in increased environmental impact. As an example, the environmental effects of plant-based protein concentrate is lower compared to that of protein isolate, as requires more extensive processing, whereas the environmental impacts of plant-based meat derived from soy protein isolate are greater than those of unprocessed chicken, pork, or beef (Berardy et al., 2019).

Due to the growing global demand for protein, insects have become an attractive source of protein for both food and feed. Among animal protein sources, insects have been recognized for their potential to be converted into food, offering a more sustainable option. In comparison to meat sources like chicken, pork, and beef, the life cycle of protein derived from mealworms emits far fewer greenhouse gases (Halloran et al., 2016). Evidently, protein content plays a significant role in determining the environmental impact of plant-based and animal-based protein products.

In summary, the analysis reveals that animal proteins have a high greenhouse gas emissions rate because livestock produce methane, and animals require energy-intensive treatment. Moreover, animal proteins account for a substantial amount of pollution, by releasing animal waste, using antibiotics, and running off chemicals from modern farming

methods. In contrast, plant proteins result in lower greenhouse gas emissions per unit of protein yield and diminished pollution rates, especially when compared with chemical fertilizers and runoff from manure (Joya-Barrero et al., 2023). Fig. 8 shows Production of Greenhouse gas emissions from different dietary protein sources.

8. Challenges in plant-based meat production and future research directions

Non-animal-based meat analogues have made significant advancements in improving nutritional profiles and texture, yet matching the flavor of traditional meat remains a persistent challenge. Strategic use of ingredients, including binding and coloring agents, is crucial for enhancing taste and visual appeal. Novel production techniques, such as extrusion, have emerged to reconfigure plant proteins into meat-like textures. For instance, heating and roasting not only enhance texture but also contribute to the generation of complex flavor profiles through Maillard reactions and caramelization processes. These methods produce flavor compounds that are typically associated with cooked meat, such as aldehydes, ketones, and sulfur-containing compounds, which impart savory, roasted, and umami notes. But the maillard reaction role depends on the extrusion conditions such as the reaction temperature, residence time in the barrel, pH, and water activity of the system (Wang et al., 2024). An analysis of the volatile flavor compounds of the extrudates indicated that dry extrusion eliminated the volatiles that originated from the native plant protein ingredients but introduced new flavors, that is, Maillard reaction products such as pyrazines, thiophenes, furans, and 1-pentanethiol (Kaleda et al., 2021). Moreover, the type of plant protein used can influence the final flavor. Legume-based proteins may have a beany flavor that requires masking or transformation through processing. On the other hand, pea protein tends to have a less pronounced inherent flavor, making it a preferable choice for flavor development. A. Sun et al. (2022), generated meat flavouring from Maillard reaction products (MRP) of wheat gluten protein hydrolysates-xylose. The results demonstrated that UV absorption and fluorescence intensity of MRPs significantly increased at 120 °C, suggesting formation of a large amount of Maillard reaction intermediates. MRPs exhibited high umami and low bitter taste at 120 °C. Therefore, understanding how different processing parameters affect flavor formation is essential for optimizing sensory attributes.

As plant-based materials primarily consist of amorphous tissues, it is essential to reconfigure them into meat-like textures. Among these techniques, extrusion stands out as a widely employed method. The texture can also be improved by optimizing process conditions or selecting appropriate plant protein sources. The challenge of achieving the right appearance, especially colour, remains a focal point in the

development of plant-based meat analogues. Meticulous research and innovation aim to ensure that these alternatives mimic the colour attributes of raw or cooked meat. For this purpose, leghaemoglobin, structurally akin to myoglobin, is integrated into meat analogues to replicate the desirable colour attribute (Shaghaghian et al., 2022). However, the manufacturing processes involved in plant-based meat analogues can sometimes inadvertently lead to a loss of nutritional value. Besides, the insufficiency of data regarding the digestibility, bioavailability, and allergenicity of some protein resources more especially insect and algae proteins, necessitates further research in this area. Despite these hurdles, the combination of cutting-edge technology and persistent innovation is expected to pave the way for plant-based meat analogues to play a pivotal role as protein sources in the future. The future of meat analogues holds immense promise as technology, innovation, and changing consumer preferences continue to shape the landscape. Advancements will involve the exploration of innovative ingredient formulations, texture modifications, and revolutionary cooking techniques. Non-animal-based meat analogues of the future might increasingly rely on simpler and more recognizable plant-, algae-, and insect-based ingredients. Furthermore, there is a prospect of hybrid products, harmoniously combining plant-based and cell-based meat, capitalizing on the strengths of both to offer sustainable and nutritious dietary choices.

Efforts to make meat analogues more accessible to individuals with food allergies will be a key consideration. As technology advances, production scales up, and costs decrease, the feasibility of making meat analogues more competitive with traditional meat products grows increasingly realistic. This transition will contribute to shaping a more sustainable and health-conscious food industry in the years to come. Fig. 9 illustrates the key challenges and potential solutions associated with using various protein sources in the production of meat analogues.

9. Conclusions

Production, sustainability and environmental concerns stemming from conventional livestock production have fueled the demand for sustainable alternatives. The livestock industry carries a multitude of adverse effects, including heightened susceptibility to zoonotic diseases and the proliferation of antibiotic resistance. Moreover, it contributes significantly to resource depletion, greenhouse gas emissions, pollution, biodiversity loss animal welfare concerns. As global awareness of these environmental and health impacts grows, there is a burgeoning interest in meat alternatives as consumers increasingly seek healthier and more ethically sound dietary choices.

Investments and dedicated research endeavors in the field of meat alternatives are poised to drive transformative advancements. These innovations aim not only to address the pressing challenges of the present but also to enhance the accessibility and affordability of meat alternatives in the future. Key plant-based proteins, such as soybeans, wheat, and peas, have emerged as the cornerstones of meat analogue formulations, while an array of other under-utilised, protein-rich plant sources worldwide holds great potential. In this comprehensive review, we have evaluated and discussed the pivotal role played by meat analogues and dissected their quintessential attributes, encompassing texture, flavour, and structural integrity. Furthermore, we have described the diverse technologies employed for meat analogue production, critically comparing their respective merits and demerits. Nevertheless, a multitude of challenges looms, ranging from issues of scale, production costs, and regulatory compliance to consumer acceptance. Therefore, ongoing research and development efforts must remain dedicated to fostering transparency, innovation, and, above all, environmental sustainability within the realm of meat alternatives. These strategic pursuits will undoubtedly pave the way for a more sustainable and ethically conscious future of food production.

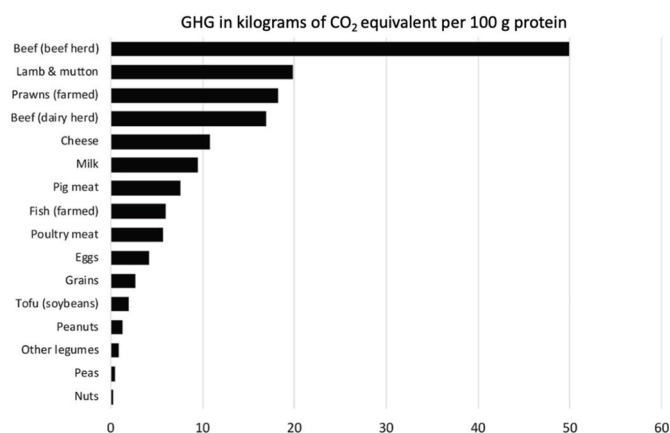


Fig. 8. Production of Greenhouse gas emissions from different dietary protein sources (Semba et al., 2021).



Fig. 9. Challenges and potential solutions in utilizing various protein sources for meat analogues production.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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Glossary

ANFs: Antinutritional factors
CATA: Check-all-that-apply
GWP: Global warming potential
HME: High-moisture extrusion
LMMA: Low moisture meat analogue
LME: Low-moisture extrusion
LPI: Lupin protein isolate
MY: Mycelium
OP: Oat protein
PDF: Pea protein dry-fractionated isolates
PPI: Pea protein isolate
RPC: Rapeseed protein concentrate
RB: Rice bran
RPI: Rice protein isolate
SPC: Soy protein concentrate
SPI: Soy protein isolate
TVP: Texturized vegetable protein isolate
3D: Three dimensional
WHC: Water holding capacity
WG: Wheat gluten
WPI: Whey protein isolate
XG: Xanthan gum