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Methodologies of meat analogue production and nutritional perspectives: A review

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Abstract. With increasing global population and environmental concerns, the development of meat analogues, including plant-based proteins and cultivated meat, has become a solution for elevating global protein production and addressing sustainability issues. North America and Europe lead the meat analogue market, driven by elevated consumer awareness of health and environmental concerns. Under the influence of western diet, the Asian market is also poised for growth. While cultivated meat industry remains immature, advancements in production and cost reductions are expected to boost its market share over time. Although research on meat analogue methodology has increased in recent years, reviews that combine production methodologies and nutritional perspectives remain uncommon. This review comprehensively analyses the production methods, nutritional values, and market prospects of meat analogues. Meat analogue production has great potential in reducing water consumption, promoting sustainability, and improving animal welfare. Meat analogue provides high protein content and balanced amino acid profile, though certain micronutrient deficiencies due to production processes. This review outlines directions for further research in meat analogue production, emphasizing the importance of enhancing production efficiency and nutritional fortification to address food insecurity and environmental damage.

Keywords: Meat analogues, plant-based protein, cultivated meat, nutrition.

1. Introduction

Protein as one of the macronutrients, plays a vital role in our health. The world's protein production failed to fulfill the global need for such large consumption. According to Alvarez et al., protein deficiency prevalence is mainly in the Afrika and Equatorial regions. Acute protein deficiency, so-called "kwashiorkor". In Afrika, kwashiorkor cases account for 50% of severe acute malnutrition (SAM) cases in countries such as Malawi and about 32% in the Democratic Republic of Congo [1].

The growing population increases the need for protein while scaling up protein production using traditional animal husbandry becomes a challenging task. 8 billion people live on Earth, sharing the air, the land, the water, and the food resources. Food and Agriculture Organization of the United Nations claimed that the world population is increasing rapidly. Statistically, the world population will reach 10 billion by the year 2050. Developed countries are now facing a decrease in birth rate, while in the Global South, the elevation of birth rate drives the need for food production [2].

Increasing protein production, especially meat, as it is a major source of protein, is important for making changes. Meat analogues are invented to imitate meaty taste without using traditional animal

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husbandry or using plant-based protein instead of actual meat [3]. The production of meat analogues could mitigate the need for animal product consumption by relieving the burden from four perspectives:

The first one is low turnover efficiency. To produce 1kg of animal protein will take approximately 100,000 litres of water. To produce 1 kg of plant protein, it only takes about 1% of water that is required to produce the same amount of animal protein [4]. Making animal protein is also efficient. The turnover of plant protein to animal protein is 6 to 1. This means livestock needs to eat 6 kg of plant protein to produce 1 kg of animal protein [4].

The second one is ecological jeopardization. The environmental impact of livestock revealed many perspectives: animal husbandry in some regions has significantly impaired the biodiversity locally. 85% of topsoil had degraded in the USA because the livestock ranching had eroded the soil and brought desertification. Following soil degradation, extensive water depletion is used on pasture and other crops. Both soil and water have negatively influenced the terrestrial and aquatic ecosystems [5]. Livestock farming and fossil fuel use are the two main sources of global methane emissions. Methane is a potent greenhouse gas with a much higher global warming potential (GWP) than carbon dioxide. Gastrointestinal fermentation in animal husbandry, especially in ruminants such as cattle and sheep. It is one of the major sources of methane emissions. Livestock plays a significant role in global methane emissions, accounting for about 40% of total methane emissions. This proportion is comparable to fossil fuel use, suggesting that livestock has a significant impact on global greenhouse gas emissions [6].

The third one is animal welfare. With a growing awareness of promoting animal welfare, the animal husbandry industry faces the dilemma of ethics and income. Promoting animal welfare usually increases the cost of livestock production. These costs may include improving the feeding environment, providing more space, reducing stress factors, and veterinary care. For livestock farmers, these additional inputs can lead to lower productivity and lower profits, which conflict with farmers' economic interests. Under the pressures from the markets, increased animal welfare cost leads to higher product prices. Product prices undermine its competitiveness in the market, especially if consumers are not willing to pay higher prices to support higher welfare standards [7].

The fourth one is health concerns. Consumers in consideration of health status would also choose to eat fewer meat products. Traditional meat products contain high levels of saturated fatty acid. Overconsumption of saturated fatty acid has been proven to increase inflammatory response and is positively linked to the risk of obesity [8]. Red meat is consumed a lot in the Western diet. High red meat consumption is correlated with an increased risk of colon cancer around 20-30% [9]. Red meat consumption also leads to a greater prevalence of cardiovascular disease mortality, up to 16% [10].

This article discusses major methodologies of meat analogue production, and their nutritional values including nutritional distinction from their ingredients or traditional meat. This article also discusses the market prevalence of meat analogues with consumers' acceptance differences. By comparing amino acid profiles and navigating production procedures, both plant-sourced and animal-sourced meat analogues are found of comparable protein content to traditional meat, however, with lower micronutrient content due to heat and pressure treatment in plant-sourced analogue, or due to deficiency in the medium for cell-culturing in animal-sourced analogue. Meat analogue industry is still developing, with a steady escalation in market share and popularity. Although drawbacks like cost and social acceptance have not yet been solved, meat analogue market is expected to prosper in the future for its green features and production efficiency.

2. Meat Analogue

Meat analogue, the so-called meat alternative, was invented to cater to the needs of both the industry and consumers' perspectives. Saving water, retaining soil quality, minimizing destruction to the ecosystem, promoting animal welfare and consumers' health, all these tags became the advantage of the meat analogue industry. In the past 20 years, the meat analogue industry quickly developed and branched [3]. To categorize meat analogue, one way is to separate them by the source of protein. Plant-based protein and animal protein are two major directions. Plant-based proteins are usually made from legumes, especially soybeans [3]. Animal proteins have a wide variety of choices from poultries to mammals,

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usually CUl from stem cells [11]. Mycoprotein made from fungi is a minor direction, but still proved to have its supporters and commercial potential [12].

Based on the structuring process, meat analogue can be concluded to two main strategies: Top-down and bottom-up [3].

Top-down refers to methods that use biopolymers shaping in an overall force field, an example will be extruding protein solution from machinery. In this process, texture is made through various approaches to improve mouthfeel [13]. Top-down products are robust and easier for scale-up production. The drawback of the top-down strategy is the difficulty of mimicking meat-like sensory properties due to the overall shaping method cannot imitate the fibrous structure of muscle and adipose tissue. Additionally, the top-down strategy is mainly used on plant-based protein, therefore more approaches are required to improve the taste [14].

Bottom-up refers to assembling a piece of meat analogue from small units. An example will be cultivated meat (CM), which is made by individual cells, and gradually grows and interconnects to a whole. This process is kept in high order as muscle tissue in animals is constructed in hierarchy and directionality. Due to the elaborate assembly of the bottom-up strategy, the texture of the product can imitate a fibrous structure, which offers finer mouthfeel than top-up products [15]. However, its level of detail makes scalable production a great challenge [3].

3. Market and Consumers

The market of meat analogue is experiencing rapid development in both volume and popularity. From aspects of economic value and market growth, meat substitutes show clear promise. The demand for plant-based meat analogue (PMBA) and CM is growing in line with increasing consumer concern about health and environmental impacts. The report predicts that the market for plant-based meat market will reach 3.5 billion dollars by 2026, with a compound annual growth rate of about 12.0% [16]. From aspect of production stability, PBMA and CM are not significantly suppressed by climate change and resource constraints because their production processes are not dependent on traditional animal husbandry. This is especially outstanding during the COVID-19 pandemic when PBMA and CM can provide more stable production and supply when traditional meat supply chains are impaired [17].

North America and Europe represent the largest markets, with a high level of consumer awareness of health and environmental friendliness. In major supermarkets and restaurants, the presence of meat analogue is common [18]. In Asia, people tend to have fewer meat products in their diet. While under the influence of globalization, the Western diet has gained more popularity among Asian people. Featured high meat consumption of Western diet widens the market in Asia, bringing an increasing demand for meat analogue for a similar reason as North Americans and Europeans [18]. Most of the meat analogue uses plant-based protein as an ingredient. Major companies including Beyond Meat, Impossible Food, and Quorn all make claims on their website, indicating their products are "healthy", or "nutritious" [19]. These health claims cater to the demand of consumers as they would like to eat food with low saturated fat and the absence of cholesterol. However, the complicated processing of meat analogue also raises concerns about whether meat analogues are all ultra-processed [20].

The progression of animal-sourced meat analogue is much slower than PBMA. Animal-sourced meat analogue production is still mostly under research. CM technologies involve an ongoing discussion from the ethics perspective. Additionally, some consumers are averse to the idea of growing flesh in laboratories. In the research done by Verbeke et al. "Yuck", and "disgust" were used in the prominence of consumers' interest [21]. Additionally, CM, although currently costly, is expected to decrease significantly as production scales up and technology matures, increasing market acceptance and economic viability.

Recent research [22] pointed out the challenge of how to scale up production has not been addressed. Searching for the best scaffold material for cells is one of the cores of achieving mass production. Developing scaffold materials that can meet the needs of cell growth while being cost-effective remains difficult. To be comparable to traditional meat, efficiency is another factor to consider. As cell meat production scales up, the bioreactor design used for proliferating the cells becomes more complex. The bioreactor must maintain stable culture conditions (e.g., temperature, pH, oxygen concentration) while ensuring continuous proliferation of high cell densities. This requires precise control over environmental parameters within the reactor and avoidance of any fluctuations in the production process that could lead to cell damage [23].

Although the CM technology still has a long way to go, Europe and the United States set out regulations for the CM industry. In 2015, Europe listed CM as a "Novel Food". Which made CM comply to the Regulation (EU) No 2015/2283. Under this regulation, any company that wants to bring cell-grown meat to market must apply to the European Commission. Furthermore, a risk assessment is required by the European Food Safety Authority (EFSA). EFSA will evaluate the scientific literature submitted, including evidence on nutritional content, potential allergens, toxicology, and so on, and ultimately the European Commission will decide whether to approve the product for sale on the market [24]. In the United States, regulation of cell-grown meat is handled by two main agencies: the Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA). The FDA is responsible for the pre-production process from cell culture to the product, while the USDA is responsible for subsequent production steps, including label management. Together, they have developed a uniform regulatory framework for cell-grown meat to ensure the food safety and legality of such products [25]. In 2020, Singapore officially put CM into the market. Singapore becomes the first and the only country to approve CM for commercial sale [26].

4. Plant-based Protein Analysis

Legumes, microalgae, and fungi are three mainstream protein sources in plant-based analogues. These plants and fungi can provide a large amount of protein with a high yield (Table 1).

4.1. Legumes

Legumes are traditional plant protein source and are made into various products offering very different sensory attribution. Legumes generally provide 20%-30% of protein. Legumes store protein mainly in the cotyledons. Legumes contain four protein types: Albumins, which are soluble in water. Globulins, which are soluble in dilute salt solutions. Prolamins, which are soluble in alcohol. Glutelin, which are soluble in dilute alkalis or acid solutions. Albumins and globulins occupied the largest portion of total protein profile [27-29].

Legumes are generally rich in essential amino acids. Legume protein isolates were found to be rich in Asp and Glu. Essential amino acid content accounts for 38.16-45.05 g in 100 g legume protein isolate [30]. The disadvantage of legume proteins is the deficiency of sulfur-containing amino acids. Total sulfur-containing amino acids in proteins from different legumes contribute 1.14-1.66 g per 100 g [30].

Soybean is the most widely used as PBMA. Soybean contains 30%-50% of protein with a balanced amino acid profile. Slight deficiency of sulfur-containing amino acids, methionine, and cysteine can be complemented by grain intake [31]. Soy protein has a high absorption rate. Protein Digestibility Corrected Amino Acids Score (PDCAAS) of soy protein isolate (SPI) is comparable to PDCAAS of egg white [32]. A recent study revealed the possibility that soy protein may be beneficial for weight loss, reduced risk of type 2 diabetes, increased bone density, and reduced risk of breast cancer [33].

4.2. Microalgae

Microalgae protein has better yields than soy protein, which makes it an outstanding choice for meat analogue. The cultivation of land plants such as beans is largely dependent on the environment and climate. Soybeans, for example, can only adapt to warm weather and must be under optimal growing conditions (average temperature 20-30 °C). The cultivation of microalgae has the potential to expand to areas and climates where traditional agriculture cannot reach, such as coastal areas. Microalgae can be cultivated in many systems, suitable for year-round harvest [34].

Microalgae contains a large amount of protein, as well as high levels of iron and calcium. Also has a high beta carotene and vitamin C. *Arthrospira platensis* (Spirulina) and *Chlorella vulgaris* both have balanced amino acid distribution. Although amino acid profiles in microalgae are not as comparable as

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legumes, they still meet the WHO/FAO/UNU standards amount of essential amino acids required [35]. Microalgae protein turnover astonishingly reaches 62 g per protein100 g for dry Spirulina and 58 g of protein per 100 g for dry Chlorella [35].

4.3. Mycoprotein (Fungi)

Fusarium venenatum is the only fungi species cultivated for PBMA. Dried *Fusarium venenatum* primarily contains high fibre (25 g/100 g), resulting in a chicken-like mouthfeel [36]. There are 11.34 g of protein in 100g of fresh *Fusarium venenatum* ingredients, and 45 g of protein in dried ingredients. PDCAAS is close to 1.0. Study shown the rich fibre content does not adversely affect mineral absorption [36].

The Glutamic acid content of dried fungi was the highest, reaching 4.7 g/100 g. However, the proportion of sulfur-containing amino acids is small, with methionine and cystine only 0.3 g/100 g. Besides protein, it also has a relatively high concentration of omega 6 and omega 3 fatty acids, respectively 4.3 g/100 g and 6.9 g/100 g [37]. Clinical studies have shown that mycoprotein can lower total and LDL cholesterol, increase satiety and reduce the glycemic response when consumed with carbohydrate-rich foods [38].

Table 1. Amino acid profile of soybean, *Spirulina*, and mycoprotein.

	Content of Amino Acid (g/ 100 g)			**Function**
	Soybean [30]	*Spirulina* Powder	Mycoprotein (Dried) [38]	
Lysine	4.7–5.8	2.26 ± 0.001 [42]	3.8	Essential for protein synthesis, hormone and enzyme production, calcium absorption, and immune function. [39]
Histidine	2.5–3.5	2.74 ± 0.03 [42]	1.6	Involves the growth, repair, and formation of blood cells. [39]
Tryptophan	0.3-1.0	0.93 [43]	0.8	Precursor to serotonin and melatonin. [40]
Tyrosine	3.9-4.9	2.03 ± 0.04 [42]	1.8	Precursor to neurotransmitters [40]
Phenylalanine	3.5-4.3	1.90 ± 0.07 [42]	2.3	Precursor to sulfur-containing compounds. [40]
Leucine	7.3-7.9	3.97 ± 0.08 [42]	3.9	Stimulates muscle protein synthesis, supports wound healing, regulates blood sugar levels, and energy regulation. [41]
Isoleucine	4.4-5.8	2.53 ± 0.04 [42]	2.4	Involving in muscle metabolism, immune function, haemoglobin production, and energy regulation. [41]
Cysteine	0.5-2.4	0.46 ± 0.006 [42]	0.4	Synthesis of glutathione, a powerful antioxidant, and skin, hair, and nails formation. [39]
Methionine	1.1-1.4	0.81 ± 0.01 [42]	1.0	Precursor to sulfur-containing compounds. Participate in metabolism, detoxification, and the absorption of zinc and selenium. [39]
Valine	4.5-5.3	2.79 ± 0.05 [42]	2.8	Used in muscle growth and regeneration, energy production, and nervous system function. [41]
Threonine	3.3-3.5	3.96 ± 0.0.1 [42]	2.5	Involving in immune function, and the formation of collagen and elastin, fat metabolism. [39]

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5. Production Methodology and Nutritional Impact

5.1. Top-Down Strategy

5.1.1. Extrusion. The extrusion method has been applied to the food industry since last century. This technology can texturize food, adding fibrous texture and anisotropic structure to enhance its sensory traits. Extrusion is widely used for making PBMAs. Plant-based proteins such as soy protein concentrate are squeezed under high humidity resulting in a meat-like texture [13]. Soy protein concentrate usually contains about 70% protein. To ensure that a suitable fibrous structure is formed during the extrusion process, mix 50% to 70% water with soy protein concentrate to form a high-moisture mixture. Before entering the extruder, the mixture needs to be pre-heated for stability during the extrusion process. Pre-heating also improves the plasticity of the protein mixture. In the extruder, the mixture is heated to a high temperature of 100 °C to 160 °C. This process causes a partial denaturation of the protein, at this point, a fibrous texture is formed. The extrusion process also involves maintaining a certain amount of time under high pressure to ensure the stability and uniformity of the protein structure [44]. Because of its low cost and massive production scale, extrusion has now become the most popular production method for the meat analogue market, technology iteration is accelerated to cater to consumers' demands [45]. However, consumers reported negative sensory aspects like uniform taste, compactness, dryness and softness still need further improvement [45].

The extrusion method involves the use of high temperature and pressure. Such a process impairs the nutritional value of the final product. In consideration of the plant-source ingredients, the vitamin C content is damaged under high temperatures, combined with high temperatures and humidity deteriorates vitamin C loss [46]. Exposure to high heat causes 20%-75% of vitamin C deactivated, thiamin (B1) 9%-70% of vitamin A deactivated and reduces folate by 50-90%. There is also a moderate loss of 20%-40% of Vitamin D [47]. Non-enzymatic browning at high pressure causes lysine loss. Prolonged boiling can lead to up to 40-50% of lysine denaturation, which makes extrusion not friendly to people who rely on PBMAS to complement lysine deficiency in grains. Water-soluble vitamins, such as riboflavin and niacin, are moderately stable, but can still be affected by high-temperature conditions. A 10%-25% reduction is common in prolonged high heat [46].

5.1.2. Shear Cell. Shear cell technology is similar to extrusion. It can be understood as an improvement in extrusion technology. In the shear cell process, an extruder is still required to squeeze out the protein mixture. An additional step is the protein mixture undergoes a well-defined shear flow in a controlled environment. The inner environment is usually controlled by a rheometer. This process involves the deformation (shearing) and solidification (cooling or cross-linking) of the protein structure to produce the desired texture. Compared to extrusion technology, the final product that went through shell cell process becomes more fibrous and with more precise control over toughness--tender or chewy [48].

Another improvement is the heating control. Compared to extrusion, shell cell technology lowers the operating temperature to 50-100 ℃ [48]. This temperature can reduce lysine denaturation and water-soluble vitamin loss. Despite the losses of vitamin C, folate, and vitamin D are still inevitable.

5.1.3. Freeze Texturization. Freeze texturing is to control the freezing rate of protein solution and the formation of ice crystals. Consolacion and Jelen introduced two freezing methods in their research [49]: rapid freezing and unidirectional freezing. In rapid freezing, protein solution is immersed directly into liquid nitrogen to freeze it instantly. This method produces many tiny ice crystals, causing the proteins to form randomly arranged porous structures. In contrast, the unidirectional freezing technique works by slowly freezing at -25 ℃ for 48 hours so that heat is removed from a single direction. This method causes the ice crystals to extend in one direction, and the proteins are pushed between the ice crystals to form parallel fibrous structures. Scanning electron microscopy (SEM) images in the literature show that rapid freezing results in a random arrangement of proteins, while unidirectional freezing results in a parallel, sheet-like structure. These parallel layers exhibit similar tissue properties to meat fibres.

Formation of ice crystals not only affects the way proteins are arranged but also determines the physical properties and sensory factors of the final product [49].

After freezing is complete, the protein structure needs to be fixed by further processing. Two methods of structural fixation are described in the literature [49]: freeze-drying and heat setting. Freeze-drying stabilizes the fibrous structure of the protein by removing water at low temperatures in 95% ethyl alcohol, ensuring that these structures remain intact after thawing. By using a heat setting, proteins are processed under high pressure and high temperature to form stronger chemical crosslinks, further enhancing the stability of the structure.

Both fixation methods are designed to ensure that protein structures formed during freezing texturing remain stable during subsequent processing and storage without disintegration or texture changes. However, the two methods impact very differently in terms of nutritional value. Freeze-drying is processed under low temperatures, and combined with the freeze texturing process, the nutrients of the ingredients are well-retained. Under low temperatures, ascorbic acid, riboflavin, α-tocopherol, and β-carotene can be preserved for a long time. Based on the research conducted by Bouzari et al. [50], frozen samples can be stored at -27.5 ℃ for 90 days to ensure a slight loss of nutrients, while fresh samples after 10 days of 2 ℃ storage experienced serious nutrient loss. Heat setting, on the other hand, has an operating temperature of 121 ℃. This process deactivates heat-sensitive vitamins, like vitamin C, folate vitamin A and vitamin D as mentioned in 5.1.1. [46].

5.2. Bottom-Up Strategy

5.2.1. Spinning. Two major types of spinning are wet spinning and electrospinning, both create fibrous products. Wet spinning was first patented by R. A. Boyer 70 years ago [51]. In wet spinning, protein concentrates are mixed with an appropriate solvent (such as an acidic solution) to form a uniform solution. The protein concentrate content used is usually between 10% and 20%, and the pH value of the solution needs to be controlled within a specific range to prevent precipitation. In the solution, there are two types of protein concentrates containing two different proteins, one forming the dispersed phase and the other forming the continuous phase. Squeezing out the protein solution via a spinneret and immersing it in a coagulation bath causes the former to be solidified and the latter is washed away. One typical example of the dispersed phase and continuous phase is soy globin and casein. The more protein concentrate content in the solution, the higher the mechanical strength of the product. Coagulation baths usually contain calcium salts or acids so to solidify protein solutions and form fibres. Fibre diameters typically range from 10-30 μm, depending on the diameter of the spinneret and the composition of the coagulation bath. Polysaccharide is added to the protein solution for better separation of two phases as well as providing stronger structural support for the final product [52].

The electrospinning method requires a polymer solution as an ingredient. Polymer solutions include biodegradable synthetic polymers such as polylactic acid (PLA) and polycaprolactone (PCL). The concentration, viscosity and conductivity of the solution directly affect the diameter and shape of the final fibre. During electrospinning, a high-voltage electric field between 10-20 kV is applied between the needle and the collector. When the polymer solution is extruded through the needle, the droplet of solution forms a Taylor cone, which then produces a polymer jet. As the solvent evaporates, the jet streams become unstable and fractures forming nanofibers. The collector is usually a conductive plate, and the polymer fibres need to be fully cured before reaching the collector. The distance between the collector and the needle (typically 10-20 cm) is one of the key parameters controlling the fibre diameter. Too short a distance may result in coarse fibres that are not fully solidified, while too long a distance may result in fibres that are too fine and difficult to control [53].

Spinning methods have great potential in nutrition retaining and cooking areas. The fibre made from spinning methods has the characteristics of high strength and food-grade quality. This made it possible to produce edible film with fibre products. The edible film has its value in food preservation as it can cover the food and cut off contact with oxygen and moisture [54]. Edible film can be fortified with

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micronutrients to complement the insufficient intake of other foods. Its high flexibility provides the culinary industry a chance to apply edible film to novel food design [54].

5.2.2. Cultivated Meat (CM). Cultivated meat, so-called cultured meat, describes the strategy of harvesting stem cells from animals and growing these cells in an *in vitro* environment. CM analogues are entirely animal-sourced, which offers a finer taste and mouthfeel closer to traditional meat than PBMAs do. Major CM technologies so far all rely on scaffolding for supporting cells growth [23]. Based on the choice of scaffold material and practical applications, scaffolding CM can be categorized into four main types: hydrogel scaffolding, microcarrier porous scaffolding, and fibre scaffolding.

5.2.2.1. Hydrogel Scaffolding. Hydrogels are primarily used in medicine. A thermosensitive hydrogel made of PEG - PLGA - PEG (polyethylene glycol - poly (lactic-co-glycolic acid) - polyethylene glycol), has proved to be effective for wound healing as well as the engraftment of muscle-derived stem cells (MDSCs) [55].

To apply hydrogel for cell cultivation, satellite cells (SC) are retrieved from animal bodies or commercial meat. Furuhashi et al. [56] used two types of hydrogels and contrasted the maturation status of contractile structure. Furuhashi et al. [56] isolated bovine muscle cells from fresh commercial beef and cultured in the hydrogel containing either collagen or fibrin-matrigel mixture The muscle cells cultured in the mixture of fibrin-matrigel were more conducive to the alignment and maturation of muscle tubes. The researchers also found when electrical pulses were applied to cultured muscle tissue, muscle contraction and the formation of myotubes were significantly enhanced.

Hydrogel is very efficient in improving water-holding capacity compared to other meat analogue production methods. Water retention of CM produced by hydrogel scaffolding reaches 90%, which is higher than traditional meat. High water holding capacity is linked with a more tender texture [56].

5.2.2.2. Microcarrier. Similar to hydrogel scaffolding, microcarriers were also primarily used in medicine. In 2013, Mark J Post and his research team invented the world’s first cultivated meat burger and conducted a taste test in London. [57].

Before SCs are loaded onto microcarriers, they must initially be cultivated on a flat plastic surface coated with hydrogel to ensure their adhesion and proliferation. Conventional culture methods use culture substrates coated with collagen, fibronectin, or laminin to promote cell attachment and growth. Then muscle cells are moved to microcarriers. Microcarriers provide a large surface area/volume ratio, which helps to expand a large number of SCs in a limited space. Microcarriers with different materials, porosity and surface structure were selected for optimizing the adhesion rate and proliferation efficiency of the SCs. For example, the adhesion effect of SCs can be improved by increasing the positive charge of the microcarrier or adjusting its hydrophilicity. In addition, larger-diameter microcarriers could better support cell attachment, but smaller-diameter microcarriers were more conducive to cell proliferation [58].

After SCs reach a certain amount, they are induced to begin differentiation by changing the medium and culture environment. The differentiation process involves the fusion of cells to form myotubes and eventually muscle fibres [58].

Microcarriers for food use only have not yet been put into production, studies also show other microcarrier mediums have potential for future food production [59]. Gelatin, collagen and alginate are suitable food grade materials for microcarrier medium [59,60].

When using non-edible microcarriers, the cells need to be separated from the microcarriers. A variety of separation techniques were explored, such as enzymatic hydrolysis, and mechanical and thermal response methods, to ensure efficient cell recovery and maintain their vitality. For degradable microcarriers, the degradation is accelerated by chemical or enzymatic solution to obtain a single-cell suspension, simplifying the separation step [58].

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5.2.2.3. Porous Scaffolding. The texturization process can create a porous and flaky structure on plant-based proteins like soy protein. Textured Soy Protein (TSP) is a by-product of soybean oil processing and has a porous structure and high protein content (53-69%) that can be used for cell attachment and proliferation. The porous feature of the material facilitates the distribution and growth of cells in the three-dimensional scaffold, providing an adequate supply of nutrients and oxygen. Adding insulin-like growth factor (IGF-1) and epidermal growth factor (EGF) significantly improved the coverage, area, and shape complexity of the muscle fibre. TSP is edible as a scaffold material, which means that it does not need to be removed in the final product, simplifying the production process [61].

5.2.2.4. Fibre Scaffolding. Fibre Scaffolding is a technology that cultures SCs on edible fibre. This technology imitates muscle fibre by attaching muscle cells to protein fibre from other sources, which can be produced by wet spinning or electrospinning. MacQueen et al. [62] made gelatin fibre by electrospinning and successfully cultivated bovine aortic smooth muscle cells and rabbit skeletal myoblasts. However, both cells appeared to have insufficient mature contractile structure. In the research, shorter fibre with a length <20 μm improves cell aggregation while longer fibre with a length >1 cm can improve muscle alignment [62].

5.2.2.5. Nutritional Perspectives on Cultivated Meat. Meats of any type are typically rich sources of protein. Meat types commonly consumed by us including beef, pork and lamb all have a balanced and abundant amino acid profile. Amino acids in animal-source protein are generally more sufficient and make consumers easier to fulfil the Recommended Dietary Allowance [63]. However, there is limited research exploring the amino acid profile of CM and other micronutrients [64]. Scaffolding material could potentially negatively affect the amino acid profile of CM. For example, collagen scaffolding often contains a high amount of glycine, which is a non-essential amino acid, high glycine content may impact the overall amino acid profile in the final product [65]. *In Vitro* environment of CM is sterile, comparing to traditional animal farming, CM is in a more controlled condition. This feature promotes the prevention of infectious diseases and bacterial growth, benefitting food safety [11]. Research also indicated some theoretic hazards as a high cell differentiation rate in CM could create cancer cells or tumour tissue. The effect of chemical addition for CM medium requires a deeper investigation. [11].

Although CM is generally considered to be similar in nutrient content to traditional meat. However, some specific micronutrients are likely to be lacking in CM. Vitamin B12 is a vitamin essential for nerve function, DNA, and red blood cell production. It occurs naturally in animal tissues and is difficult to obtain from plant foods. The cells used for CM do not naturally produce vitamin B12 because it is usually synthesized by bacteria in the animal's intestine [15]. To achieve the nutrient levels of traditional meat, vitamin B12 needs to be added to the final product. Traditional meat is rich in other B vitamins, such as niacin, riboflavin and folate, which are essential for energy metabolism and maintaining healthy cells. Like vitamin B12, CM needs to be fortified to get the same amount of these nutrients.

CM is produced under high oxygen environment; therefore, CM does not require as much heme iron as traditional meat to transport oxygen and support body activity. In CM, iron content is generally lower. Zinc and selenium are important for immune function, DNA synthesis, and antioxidant stress. In traditional meat, livestock is obtained from forage. These nutrients are not directly available to cells in CM. Traditional meat contains a variety of bioactive compounds, such as creatine, taurine, and carnosine, which have been linked to muscle function, brain health, and antioxidant properties [15]. These compounds occur naturally due to the metabolism of animals that are deficient in CM products. Fortification is essential for CM to remain the same micronutrient profile.

6. Conclusion

Considering the escalating global population and the constraints of limited resources, the advancement of plant-based protein and cultured meat technologies shows great value in enhancing food security and sustainability. This paper has explored the production methodologies, nutritional attributes, and market

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acceptance of these meat analogue technologies, highlighting their critical role in addressing global challenges.

The production technologies for plant-based protein and cultivated meat are highly diverse in terms of developmental trends. Top-down strategy, especially extrusion technology, is a predominant technology in producing plant-based protein, protein solution under high temperature and pressure to form fibrous structures akin to meat. Despite its extensive application, the degradation of essential nutrients, such as some vitamin B and lysine, remains a critical technology for the meat analogue market. Concurrently, bottom-up strategy, cultivated meat production through cellular agriculture not only conserves substantial quantities of land and water but also diminishes reliance on animal farming. Cultivated meat can largely imitate meat flavour. The selection of scaffolding materials, including hydrogels and microcarriers, plays a vital role in influencing cellular growth and the nutritional composition of the final product.

Nutritionally, both plant-based proteins and CM offer substantial benefits, including rich amino acid profiles, and reduced or customized fat content, which collectively aid in mitigating risks associated with cardiovascular diseases and obesity. Specifically, sources of plant protein such as soybean, microalgae, and mycoprotein are extensively used as ingredients for meat analogues due to their balanced amino acid profiles. However, CM, while nutritionally similar to conventional meats in flavour and texture, confronts challenges regarding the synthesis of bioactive substances and nutrients in animals' forage. More research is required for further fortification methods in production technology.

The market acceptance of meat analogues is largely driven by increasing consumer awareness of health and environmental concerns. North America and Europe lead the market for both plant-based proteins and cultivated meat, with growing consumer demand for sustainable and nutritious alternatives to traditional meat. In Asia, the popularization of the western diet, coupled with high economic growth, creates opportunities for the expansion of meat analogue market. However, concerns about food safety, sensory quality, and ultra-processing remain barriers to widespread consumer acceptance. Overcoming these concerns will require ongoing improvements in production technology and clear communication of the health benefits and environmental advantages of these products. From the perspective of economic value analysis, with the progress of production technology and the realization of economies of scale, the production cost of meat substitutes is gradually reducing, making these products more competitive in price. In addition, increased government and corporate investment is also driving the development of this industry, such as through research and development subsidies and marketing activities to enhance consumer awareness and acceptance of novel foods.

In conclusion, the development of plant-based meat analogues and cultivated meat technologies represents a vital step forward in addressing global food insecurity and environmental damage. Further innovations in production efficiency, nutrient fortification, and large-scale manufacturing will be necessary to meet the demands of a growing population. Additionally, increasing consumer education and addressing bias about meat analogues will play a critical role in driving market adoption. Together, with the combination of technological advancements and strategic market positioning, the meat analogue industry will thrive and contribute significantly to a more sustainable and secure global food system.

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