Alternative Proteins

Subjects: Food Science & Technology

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Definition

Alternative Proteins include cultured meat, plant-based meat, insect protein and single-cell protein. Here, the technological, safety and environmental aspects of these protein sources are described.

1. Introduction

Global population is projected to reach 9.8 billion in the year 2050 [1]. This population growth entails a projected livestock production of 455 million tons in 2050 [2], which is 40% higher than the reported number in 2019 [3]. Currently, livestock production contributes to 14.5% of anthropogenic greenhouse gas (GHG) emission [4]. In particular, livestock production releases methane and nitrous oxide gases, which have higher global warming potential than carbon dioxide [5]. To avoid potential catastrophic events, global temperature increase should be maintained within 1.5 °C of the pre-industrial levels [6]. Considerable requirements for water and land further contribute to the environmental footprints of livestock production [7][8].

Mitigation efforts include improved feeding practices for better forage digestibility, manure management and diversification of crop and animal varieties [9][10]. Nevertheless, climate issues are a matter of great urgency and more radical solutions may be necessary to ensure food availability in an environmentally sustainable manner. Thus, the concept of alternative protein arises as an attempt to substitute conventional meats with other protein sources that require less intensive production means.

Several examples of novel protein sources are cultured meat, plant-based meat, insect protein and single-cell protein, which have gained interests from researchers and the food industry in the past few years.

2. Technological Aspect

Cultured meat, also known as in vitro, lab-grown or cell-based meat, is derived from animal stem cells that are cultivated in controlled settings. Currently, the two main stem cells considered to be the most suitable for culturing meat are embryonic stems cells or satellite cells [11]. The main steps involved in the production of cultured meat include the isolation of stem cells from an animal biopsy, followed by the proliferation and differentiation of these isolated stem cells into desired tissues (for example, skeletal muscles) in a cell culture medium [11][12]. In the process, the growing cells can be attached to scaffolding materials, such as collagen-like gel polymers, which serve as a support network for the tissue development [11][12] —potential polymers to be used as scaffolds are listed elsewhere [13]. Trecapitulation of complex meat structures via tissue engineering needs to include skeletal muscles (myogenesis), extracellular matrix (fibrogenesis), microvascular networks (vascularization) and intramuscular fats (adipogenesis) [14]. Thus, there are practical advantages of using pluripotent stem cells, especially the induced pluripotent stem cells (iPSC) derived from adult cells [15][16].

Texturized vegetable proteins (TVP) can be used as a potential replacement of conventional meat and are commonly derived from soy proteins [17], or to a lesser extent, from wheat glutens [18][19] and legume proteins (for example, pea and chickpea) [20][21]. Currently, plant-based meats are mainly produced through thermoplastic extrusion [19][20][22][23][24]. This process can be categorized based upon the amount of water added, i.e., low moisture (20–35%) or high moisture (50–70%) [25]. Both product types are made in three main steps: (1) pre-conditioning of the raw materials outside of the extruder; (2) heating and compression inside of the extruder; (3) cooling of the die and processing of the final product (for example, cutting to desired pieces) [26]. Shear technology has also been used to structure vegetable proteins [27].

Insects have been a part of the human diet for centuries, particularly in Asia and Africa [87]. According to the Food and Agriculture Organization of the United Nations (FAO), there are over 1900 insect species consumed around the world [28]. This practice of eating insects, also known as entomophagy, is sustainable due to the high amounts of protein and polyunsaturated fatty acid contained in edible insects, although there are variations across species [29][30][31][32]. Insects are also more effective in converting feed into edible body mass than farm animals [33]. These have made them an attractive option for expanded production to improve global food security. Most edible insects are harvested from the wild, but they can also be semi-domesticated through habitat manipulation or reared in farms for a mass-scale production [28][34]. Similar to other animals, insects require macronutrients (lipids, proteins and carbohydrates) and micronutrients (essential sterols and vitamins), which can be derived from animals, plants and yeast [35]. In particular, polyunsaturated acids, essential amino acids and sterols must be supplied in the feeds, given that insects lack the ability to synthesize these compounds in sufficient amounts [36]. In addition to adequate nourishments, rearing conditions (for example, temperature, humidity and population density) need to be optimized [37].

Single-cell proteins, also known as microbial proteins, are commonly derived from microalgae, fungi or bacteria. In their review article, Ritala et al. summarized available studies on potential fungal, microalgal and bacterial species for application in the production of single-cell proteins, including patents from the years 2001 to 2016 [38]. These include green algae (*Chlorella vulgaris*) , *Haemotococcus pluvialis*, *Dunaliella salina* and spirulina (*Arthrospira maxima* or *Arthrospira platensis*) [39], *Fusarium venenatum* A3/5 (previously known as *Fusarium graminearum* A3/5) [40] and *Methylcoccus capsulatus* [41][42].

3. Safety Concerns

Briefly, cultured meat grown in fetal bovine serum-based media can be exposed to viruses or infectious prion, in addition to other safety risks associated with the use of genetic engineering. Plant-based meat may contain allergens, anti-nutrients and thermally induced carcinogens. Microbiological risks and allergens are the primary concerns associated with insect protein. Single-cell protein sources are divided into microalgae, fungi and bacteria, all of which have specific food safety risks that include toxins, allergens and high ribonucleic acid (RNA) contents. The environmental impacts of these alternative proteins can mainly be attributed to the production of growth substrates or during cultivation. Legislations related to novel food or genetic modification are the relevant regulatory framework to ensure the safety of alternative proteins. Detail explanations are provided in the original version of this article.

4. Environmental Impact

The environmental impact of cultured meat may vary depending on the growth medium used (Table 1). Regardless, current data suggest that the theoretical greenhouse gas emission, water use, eutrophication and land use in culturing meat are lower than conventional meat production, although cultured meat is still more energy intensive [43][44]. Similar to cultured meat, the environmental impact of insect-based food production depends on the type of feed used [45][46][47], with nutritious waste-based feeds being the most environmentally friendly [46].

Table 1. Life cycle analyses (LCA) of alternative proteins.

Protein Type	**Energy Use (MJ/kg)**	**GHG Emission (kg CO$_2$-eq/kg Product)**	**Water Use or Eutrophication** [a]	**Land Use (m^{2}a/kg)** [b]	**Reference**
Cultured meat					
Minced beef [1]	26–33	1.90–2.24	0.36–0.52 m^3/kg meat (W)	0.19–0.23	[43]

Protein Type	Energy Use (MJ/kg)	GHG Emission (kg CO -eq/kg Product)	Water Use or Eutrophication	Land Use (m a/kg)	Reference
CHO [2]	106	7.5	7.9 g PO_4-eq/kg meat (E)	5.5	[44]
Plant-based meat					
Beyond Burger®	54.15	3.35	28.84 m^3/kg meat (W)	3.97	[48]
Impossible Burger®	NA	3.5	0.11 m^3/kg meat (W); 1.3 g PO_4-eq/kg meat	2.5	[49]
Insect protein					
Mealworm (T. molitor and Zophobas morio)	33.68	2.65	NA	3.56	[45]
Black soldier fly (H. illucens)	21.20–99.60	1.36–15.10	NA	0.032–7.03	[46]
Cricket (G. bimaculatans and A. domesticus)	NA	2.29	0.43 m^3/kg cricket (W); 0.00047 kg P-eq and 0.020 kg N-eq/kg cricket (E)	NA	[47]
Single-cell protein					
Spirulina tablets (A. platensis)	7.88–12.7	5.05–7.71	0.015–0.022 kg N-eq/kg tablet (E)	NA	[50]
Micoalgal protein (A. platensis)	1225.6–3338.3	78.1–196.3	3.2–3.3 m^3/kg protein meal (W); 49.2–85.3 kg N-eq/kg protein meal (E)	1.7–4.3	[51]
Microalgal protein (C. vulgaris)	217.1–4181.3	14.7–245.1	0.3–3.9 m^3/kg protein meal (W); 40.6–105.3 kg N-eq/kg protein meal (E)	1.9–5.4	[51]
Mycoprotein	60.07–76.8	5.55–6.15	NA	0.79–0.84	[52]
Bacterial protein (Cupriavidus necator)	NA	0.81–1	0.0001–0.0038 m^3/kg protein (W); 0.000333 kg P-eq/kg protein (E)	0.029–0.085	[53]
Bacterial protein (hydrogen-oxidizing bacteria)	200	8	2.5 m^3/kg protein (W); 0.0025 kg P-eq/kg protein and 0.00035 N-eq/kg protein (E)	0.8	[54]

GHG, greenhouse gas; NA, not available. [a] Water use (W) is expressed in volumetric unit (m^3 or L/weight of product), whereas eutrophication (E) is a measure of the amount of contaminants released into freshwater or marine environments (g contaminant/weight of product). [b] Land use is expressed in annual area occupation (m^2a). [1] Cyanobacterial hydrolysate was assumed as the growth medium. [2] LCA was conducted based on the proliferation of Chinese hamster ovary (CHO) in a growth medium mainly comprising basal medium and soy hydrolysate.

Available life cycle analyses on two commercial plant-based meats—namely, Beyond Burger® and Impossible Burger®—suggest that these products are more environmentally sustainable than conventional meats, as measured by their energy use, carbon emission, land use, water use and eutrophication. The environmental impact of these plant-based meats mainly occurs during the raw ingredient production [48][49].

Hydrogen-oxidizing autotrophic bacteria, such C. necator, can be turned into a sustainable protein source with a lower environmental impact than animal-based meat, including beef, fish and poultry [54]. As electricity consumption is the main driver of bacterial protein production, energy sources should be optimized for a mass-scale production [53][54]. Similarly, the cultivation of microalgae is the most energy intensive stage, and thus it contributes towards the majority of the environmental footprints associated with microalgal protein production [50], particularly when an open raceway pond is used [51].

Smetana et al. conducted a comparative study of different alternative proteins and found that cultured meat had the highest environmental impact (carbon emission and water use), as compared with mycoprotein and insect-based protein, including when caloric and protein contents were considered. However, the production of insect-based protein required the largest amount of land occupation, relative to mycoprotein and cultured meat [52]. Consistent with our data (Table 1), another study by Smetana et al. reported that microalgal proteins were more energy intensive, and thus had a higher carbon emission than other protein sources, including cultured meat, insect, yeast and bacteria [55]. Interestingly, the data collated in this review also indicate that cultured and plant-based meats have lower eutrophication potential than insect and single cell proteins (Table 1). To our knowledge, the two reports by Smetana et al. [52][55] are the only studies that directly compared the environmental impacts of these alternative proteins, and thus more research is required to establish a firm scientific framework for this issue. It is noteworthy that as functionalities of different food types can vary (for example, protein content or nutrient availability), direct comparison of the environmental impacts of different alternative proteins should be conducted with prudence.

5. Future Outlook

Alternative proteins are a growing industry, and thus the global food sector should initiate collaborative efforts to ensure the safety of foods in this category. The main focus of these efforts should be to maintain food safety in a mass-scale production, including aspects related to allergens, pathogens, chemical contaminants and the environmental implications during production scale-ups.

There is a lack of research on the safety of cultured meat, with most studies focusing on technological improvements for better production means. Infectious prion and viruses are potentially the main hazards related to cultured meat production using serum-based media. Thus, future developments of methods for removing these contaminants are warranted, such as the use of hollow fiber anion-exchange membrane chromatography to remove prion from large volumes of cell culture media [56]. Concerns about the introduction of foreign genes, such as during the conversion of somatic cells into iPSC, may be circumvented by the use of small molecules as an alternative cell reprogramming system [57]. In the current literature, it appears that antibiotics are not used in the production of cultured meat, primarily based upon the notion that this alternative protein is produced in a highly controlled and closely monitored environment [58][59]. However, to our knowledge, there have not been any studies addressing this issue using verifiable data, and thus we encourage the scientific community to investigate this issue further, including through the provision of assessments of the safety measures used to control biological contaminants in cultured meat without antibiotics.

Available data suggest that plant-based meat may contain allergens, anti-nutrients or traces of glyphosate, although activities of these compounds may be reduced by heat treatments. In the future, there is also a need for discussion of the health implications of extensive processing (i.e., ultraprocessed) involved in the production of plant-based meat, including potential development of carcinogens during the thermal treatments.

Allergens are one of the primary safety issues associated with the consumption of insects, but the clinical significance of this is yet to be established. Future research can aim at identifying the types of allergen present in different edible insect species, and subsequently assessing their health effects across demographics, i.e., by age, allergy status, ethnicity, etc. Microbiological content of insects also varies with species, and future mass-scale production of edible insects would require careful selection of those species harboring bacteria communities that are less pathogenic to humans.

Toxins pose a health risk related to single-cell protein. In microalgae, this is primarily due to environmental cross-contamination, which indicates the importance of choosing appropriate cultivation reservoirs. For mycoprotein, allergens are the main hazard, and future research is necessary to identify the risk factors associated with mycoprotein allergies. When bacteria are used as single-cell protein, careful selection of non-pathogenic bacterial strains is paramount.

In the current literature, the regulatory framework for novel foods has been described based upon food standards in Europe and the USA. As alternative proteins are a global strategy to mitigate climate and environmental issues, the scientific community should expand the scope of the discussion to include food standards in other parts of the world. For example, Australia-New Zealand Food Standard Code Standard 1.5.1 describes the pre-market assessment criteria for novel foods intended for sale in Australia and New Zealand [60], or Schedule 25 lists the approved novel food products, including several that are derived from microalgae [61]. Soy leghemoglobin has also been approved in these two countries [62].

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Keywords

alternative proteins;cultured meat;plant-based meat;edible insects;single-cell protein;novel food;environmental issues;food safety

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