CHAPTER 15

TRENDS IN CELL-BASED SEAFOOD: THE USE OF BIOTECHNOLOGY FOR NUTRITION AND SUSTAINABILITY

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ABSTRACT: In recent years, the animal protein industry has grown significantly due to population expansion. However, this has increased animal waste generated during meat production processes. To address this issue, cellular agriculture is becoming a promising biotechnological approach to sustainable production. The aim is to reduce animal dependency and suffering by developing cell-based protein sources, derived from animal tissues through cell cultures. This technology is rapidly advancing to meet the challenge of feeding the world's growing population a diet that is healthier, sustainable, animal-free, and environmentally friendly, and that generates minimal or no animal waste. This mini-review explores the advances and challenges in cell-based seafood production, highlighting the relevant methodologies for obtaining them from commercially important marine species and underscores the importance of developing alternative marine proteins with nutraceutical properties for the future.
KEYWORDS: alternative protein, cultivated meat, cellular agriculture, innovation.

TENDÊNCIAS EM FRUTOS DO MAR À BASE DE CÉLULAS: O USO DA BIOTECNOLOGIA PARA NUTRIÇÃO E SUSTENTABILIDADE

RESUMO: Nos últimos anos, o setor de proteína animal cresceu significativamente devido à expansão populacional. No entanto, isso resultou em um aumento dos resíduos animais gerados durante os processos de produção de carne. Para resolver esse problema, a agricultura celular está se tornando uma abordagem biotecnológica promissora para a produção sustentável. O objetivo é reduzir a dependência e o sofrimento dos animais por meio do desenvolvimento de fontes de proteína baseadas em células, derivadas de tecidos animais por meio de culturas de células. Essa tecnologia está avançando rapidamente para enfrentar o desafio de alimentar a crescente população mundial com uma dieta mais saudável, sustentável, livre de animais e ecologicamente correta, e que gere o mínimo ou nenhum resíduo animal. Esta mini-revisão explora os avanços e desafios na produção de frutos do mar com base em células, destacando as metodologias relevantes para a sua obtenção originada de espécies marinhas comercialmente importantes e ressalta a importância do desenvolvimento de proteínas marinhas alternativas com propriedades nutracêuticas no futuro.
PALAVRAS-CHAVE: proteína alternativa, carne cultivada, agricultura celular, inovação.

INTRODUCTION

The escalating global demand for animal-derived protein exacerbates the strain on ecosystems and biodiversity (FAO, 2018). Universal meat production significantly contributes to greenhouse gas emissions, with a substantial portion attributed to deforestation for grazing land (Steinfeld *et al.* 2006). Traditional agriculture is currently confronted with a formidable challenge. The consumption of animal protein and population growth exert pressure on biodiversity and deplete natural resources, thereby jeopardizing food security (Eibl *et al.* 2021; Mc Carthy *et al.* 2018).

Overfishing threatens marine biodiversity and food security, resulting in declines in seafood biomass and endangering animal populations with extinction (Palomares *et* *al.* 2020). Over 15 years of exploitation, industrial fishing and fisheries are estimated to have reduced the ocean's biomass content by 80% (Myers; Worm, 2003). The demand for seafood is expected to rise due to its high content of quality protein, vitamins, trace elements, polyunsaturated fatty acids and minerals (Hassan *et al.* 2020).

Cultivated meat and seafood represent novel foods with the potential to offer an ethical, sustainable, and healthy protein source (Ong *et al.* 2021). Cell-cultured seafood production has the potential to alter several fundamental parameters considered constant in food production, including the generation of inedible excess tissue such as bones, blood, and skin, which are often discarded, leading to a negative environmental impact (Arvanitoyannis; Kassaveti, 2008). In addition to reducing environmental impact, cell-based food products can foster the development of local and autonomous markets by producing indirect environmental benefits. It can also shorten cycle times by streamlining supply chains and reducing production times from months to weeks to create functional foods (FAO; WHO, 2023).

As aquaculture transitions towards more intensive, controllable, and efficient systems, cell-cultured seafood production offers a new option to address the challenges associated with industrial aquaculture and marine fishing. This mini-review explores the opportunities and challenges in cell-based seafood production, addressing aspects such as marine cell culture, natural marine muscle tissue, and considerations regarding marine animals. We aim to summarize the trends and frontiers of cell-based food applied to seafood and to provide an overview of relevant methodologies for obtaining cells from commercially important marine invertebrate species.

SEAFOOD AS A SOURCE OF NUTRACEUTICALS

Nutraceuticals are substances, either in whole or in part, delivered as dietary supplements or ingredients clinically proven to offer benefits, including prevention and treatment of diseases. Marine nutraceuticals specifically pertain to compounds derived from sea (Ande *et al.* 2017). These compounds encompass oils (from fish, algae, seal blubber, and shark liver), which are rich in long-chain omega-3 polyunsaturated fatty acids, as well as shark cartilage, chitin, chitosan, and associated products, enzymes, peptides, protein hydrolysates, vitamins A, D and E, and other products (Alasalvar; Shahidi; Quantick, 2002). These marine nutraceuticals possess numerous unique features not found in nutraceuticals obtained from terrestrial resources, thus attracting increasing attention (Suleria *et al.* 2015).

Interest in seafood and other marine-derived compounds arises because most aquatic organisms have inherent mechanisms to survive hostile oceanic environments, including varying salinity, pressure, temperature, and illuminations. Most marine organisms produce several secondary metabolites that, while not directly involved in central physiological functions, contribute to their survival. They synthesize novel compounds with interesting bioactivities, facilitating adaptation to these conditions (Venugopal, 2018). Nutraceuticals derived from seafood have already been recognized for their beneficial effects on human health, attributed to various physiological functions (McManus; Newton, 2011). Studies indicate that consuming seafood can reduce the risk of heart attack, stroke, obesity, and hypertension (Stanek *et al.* 2023; Giosuè *et al.* 2022; Riccardi, 2022; Anand *et al.* 2015). Seafood is low in saturated fat and higher in "heart healthful" polyunsaturated fat, including omega-3 fatty acids (Reames, 2012).

The Bivalvia class, which represents the shellfish, constitutes a significant food source and is part of the traditional diet of numerous cultures (Tabakaeva; Tabakaev, Piekoszewski, 2018). The nutritional value of these mollusks includes proteins and amino acids of high biological values, B-complex vitamins, saturated long-chain fatty acids, and minerals (Wright; Fan; Baker, 2018; Willer; Aldridge, 2020). The levels of saturated amino acids, omega-3 fatty acids, potassium, sodium, iron, and chlorine are higher in saltwater bivalves compared to freshwater bivalves (Moniruzzaman *et al.* 2021). Considering such nutritional composition, regular consumption of mollusks could improve immunity and reduce the risk of developing diseases (Chakraborty; Joy, 2020). The shells, soft tissues, and mucilage of mollusks are used in the pharmaceutical and food industry to develop medications for the treatment of various illnesses (Khan; Liu, 2019; Pissia, Matsakidou, Kiosseoglou, 2021; Lobine, Rengasamy; Mahomoodally, 2022).

Among the critical seafood, decapod crustaceans, which include lobsters, shrimps, and crabs, stand out for their significant role in the human food supply and the global economy (Mahmood Ghafor, 2020; FAO, 2022). Besides their ecological importance to the environment, decapod crustaceans are relevant for human health, providing abundant protein and micronutrients for nutrition (Behringer; Duermit-Moreau, 2021). The high commercial value of these animals attracts agribusiness, food industries and the marine ornamental business (Calado *et al.* 2003).

Aquaculture farming and fishing ensure a quality protein source for human consumption. Fish is a rich source of essential nutrients, including highly digestible proteins, vitamins A, and D3, trace minerals as iodine and selenium, and n-3 long-chain polyunsaturated fatty acids and its consumption is generally regarded as part of a healthy dietary pattern (Ramalho Ribeiro *et al.* 2019). In addition to their high nutritional value, fish proteins have functional properties such as water-holding capacity, gelling, emulsification, and textural properties. These characteristics play crucial roles in determining the textural attributes of these products, making them important quality parameters and strategic tools for cell-based sustainable biotechnology (Pal *et al.* 2018).

ADVANCES IN CELL-BASED SEAFOOD

Given livestock production's significant adverse impacts, establishing an in vitro meat production system is increasingly justified (Datar; Betti, 2010). Consumer demand for cellular meat production primarily arises from concerns about the environment and animal welfare. At the same time, secondary considerations include consumer and public health aspects of animal production and food security (Warner, 2019).

Producing meat through tissue engineering and cell culture is not new. Initially, early attempts were concentrated in universities and other research units. Before Mark Post showcased the world´s first so-called lab-grown hamburger in late 2013 (Kupferschmidt, 2013), NASA had invested in producing edible fish muscle protein *in vitro*. The goal was to send high quantities of nutritional food to space with the least possible volume. To achieve this, researchers isolated muscle cells from the dorsal muscle of *Carassius* fish as an initial step (Benjaminson; Gilchriest; Lorenz, 2002).

Following the initial academic efforts, the concept of cell-based meat garnered attention in the food industry. Consequently, various startups related to the production of cell-based meat or the development of technologies enhancing its manufacture emerged, with many focusing on seafood **(Table 1)**. While some focus has been on the biology and engineering required to optimize the manufacturing process, most of the debate has revolved around cultural, environmental, and regulatory considerations (Faustman *et al.*, 2020). As startups began to develop prototypes of structured foods, new regulatory concerns emerged. The first governmental approval for the commercialization of cell-based meat was granted by the Singapore Food Agency in late 2020, permitting *Eat Just*, a startup based in the United States, to sell cell-based chicken in Singapore (Southey, 2022).

Company's Name	Focus	Location	Year of Foundation
Fineless Foods	Fish	USA	2016
Wild Type	Fish	USA	2016
BlueNalu	Seafood	USA	2017
Avant Meats	Seafood	Hong Kong	2018
Shiok Meats	Seafood	Singapore	2018
ArtMeat	Beef and Seafood	Russia	2019
Bluu Biosciences	Seafood	Germany	2020
Cultured Decadence	Seafood	USA	2020
Magic Caviar	Caviar	Amsterdam	2020
Umami Bioworks	Seafood	Singapore	2020
Wanda Fish	Fish	Israel	2021
Mermade Seafood	Seafood	Israel	2021
Sustineri Piscis	Fish	Brazil	2023

Table 1: List of start-ups focusing on the production of cell-based seafood

Market entry of cell-based food products may require authorization at various levels, encompassing processes such as a food safety assessment of the cell-based food product, approval of planned and implemented quality controls, assurance protocols for the production process, and adherence to approved labeling requirements for the products. The essential elements for a practical regulatory framework for cell-based food are still considered in many countries (FAO, 2022). In the Brazilian regulatory context, the Good Food Institute Brazil (GFI) has launched a regulatory study to identify potential adjustments in the current regulatory frameworks. Scientifically grounded arguments support this endeavor and involves engaging the country's regulatory agencies (GFI, 2022). Recently, GFI Brazil published a Food Safety Plan for a cultivated meat burger, contributing to assessing safety aspects in cultivated meat production through applying the Hazard Analysis and Critical Control Points (HACCP) approach (Sant'Ana *et al.* 2023).

The recent evolution of cell culture techniques that facilitate the growth of edible animal tissue *in vitro* represents an example of potentially disruptive technology with many exciting aspects to consider (Van der Weele et al., 2019; Stephens; Sexton; Driessen, 2019). With the current advancement in technology, lab-grown meat, also known as cultured meat, is expected to significantly impact the food market in the future (Ismail; Hwang; Joo, 2020).

CHALLENGES IN CELL-BASED SEAFOOD

The absence of fish and marine invertebrate cell lines means that cell culture research is conducted using primary cells isolated from these animals. A primary cell culture is initiated directly from the tissue (Jedrzejczak-Silicka, 2017). The isolation process involved in primary culture allows for precise control over hormonal, substrate, and physical conditions, which can influence cell and tissue function (Hightower; Renfro, 1988). Muscle and fat cells are crucial cell types aiming for the final product. As some of these cells can be hard to isolate, precursors of these cell types, such as satellite cells, fibro-adipogenic progenitors, pre-adipocytes, and mesenchymal stem cells, become strong candidates to be used as sources for the development of a cell-based seafood prototype (Bomkamp *et al.* 2023).

Isolation and Primary Culture

The techniques for obtaining primary cell cultures from seafood are similar to those used for mammals and other animals. Initially, the animal is sterilized with ethanol and anesthetized, after which the tissue of interest is removed under sterile conditions and subjected to a series of antibiotics to prevent microbial contamination. Subsequently, the desired tissue undergoes dissociation methods, such as enzymatic dissociation (Plotnikov, Karpenko; Odintsova, 2003; Oestbye; Ytteborg, 2019) or mechanical dissociation (Van der Merwe *et al.* 2010), to obtain a single-cell suspension for seeding in an appropriate culture medium.

Alternatively, the explant method can be employed instead of the single-cell suspension. In this case, no enzyme is used, and the original tissue is minced into smaller pieces, which are then placed in culture flasks. Cells begin to migrate out of the tissue pieces and adhere to the surface of the culture flask (Freshney, 2010; Potts *et al.,* 2020). Observations have been made of cell migration and growth from heart explants of the Indian Mud Crab *Scylla serrata* for over three weeks, demonstrating adherent cells with round, epithelioid-like, and fibroblastic morphologies (Sivakumar *et al.* 2019).

Previous studies have reported protocols for cell sorting to establish primary monogenic cultures. Techniques utilizing density gradient media to separate cells by density have yielded significant results (Gong *et al.* 2008; Odintsova; Dyachuk; Nezlin, 2010; Nogueira *et al.* 2013), particularly for shrimp hepatopancreatic cells (Toullec *et al.* 1992). Koiwai *et al.* (2019) recently isolated crustacean hemocytes using lectins and magnetic-activated cell sorting (MACS).

Over the past 30 years, researchers have attempted to create primary cultures of various crustacean species, with shrimps being the most commonly studied. Methods have been described for culturing shrimp ovaries, lymphoid tissues, cardiac, nerve, hematopoietic, hepatopancreatic, and epidermal cells (Nadala; Lu; Loh, 1993; Luedeman; Lightner, 1992; Tapay *et al.* 1995; Chen; Wang, 1999; Kasornchandra *et al.* 1999; Maeda *et al.* 2004; Anoop *et al.* 2021). Other crustaceans, such as crabs and lobsters, have also been the focus of primary cell culture research (Fadool; Michel; Ache, 1991; Stepanyan, 2004; Li; Shields, 2007; Sashikumar; Desai, 2008; Deepika, Makesh; Rajendran, 2014; Sivakumar *et al.* 2019). Among the primary tissues developed, cardiac tissue (Owens; Smith, 1999) and ovaries (Fraser; Hall, 1999) have shown more promising results, remaining viable in culture for more extended periods.

Various types of bivalve mollusk tissues have been experimented with to initiate primary cell cultures. Heart (Cecil, 1969; Wen; Kou; Chen, 1993), mantle (Perkins; Menzel, 1964), digestive glands (Le Pennec; Pennec, 2001), and gills (Gómez-Mendikute *et al.* 2005; Cornet, 2006) have shown substantial results in the growth of primary cell cultures of bivalve mollusks. Similarly, embryonic tissue (Boulo *et al.* 2000) and hemolymph (Ji *et al.* 2017) have also demonstrated significant results. Among these tissue sources, cultures from embryonic tissues show better potential for proliferation than cells from adult tissues (Odintsova; Khomenko, 1991).

Recent research has successfully cultivated adductor muscle cells of scallop *Patinopecten yessoensis,* obtaining fibroblast-like cells with multiple filopodia, similar to precursors of mature muscle cells in mammals. This was achieved using tissue explant methods and supplementing the medium with adductor muscle extract, fetal bovine serum, and supplements for insect cell culture (Suzuki *et al.* 2021).

In vitro culture conditions for marine animal cells

Concerns regarding the culture conditions of mollusk cells have been addressed over the years. The growth medium commonly used for the cultivation of marine bivalve cells typically consists of a formulation of L-15 medium (Chen; Wang, 1999; Ladhar-Chaabouni *et al.* 2021) supplemented with soluble factors to enhance cell viability (Domart-Coulon *et al.* 1994). Adding taurine, an amino acid found in bivalve hemolymph, to the medium regulates osmolarity and improves cell viability (Lange, 1963). Medium osmolarity, pH, and incubation temperature are adjusted based on the specific animal species and body part. Generally, the growth medium´s osmolarity must be like hemolymph's (Odintsova; Khomenko, 1991), typically ranging between 760 to 1100mOsmol for bivalves. The temperature of incubation is also a variable parameter. While low incubation temperatures reduce the risk of culture contamination, temperatures below 15º C do not contribute to cell migration.

The substrate to which cells adhere has significant effects on culture viability. Attached cells often exhibit increased metabolic activity (Ben-Ze'ev; Farmer; Penman, 1980). A desirable substrate promotes cell attachment and spreading in vitro, and considering that muscle cells are anchorage-dependent, selecting a compatible substrate is vital for bivalve muscle cells. It has been demonstrated that poly-D-lysine with a molecular weight exceeding 100 kDa promotes conditions for attachment of bivalve heart muscle cells in vitro (Buchanananan *et al.* 1999).

Fish cell culture protocols are very similar to other established animal cell cultures, with some adaptations regarding incubation temperature and medium osmolality specific to different fish species (Fernandez *et al.* 1993). Commonly used growth media are Eagle's Minimum Essential Medium (EMEM), Leibovitz Medium L-15 or Medium 199 (Fryer; Lannan, 1994). According to Wolf and Ahne (1982), the more commonly used and elaborate media include vitamins and amino acids, often supplemented with fetal bovine serum (FBS). FBS is a joint supplement used in various protocols for animal cell cultures, containing a mixture of amino acids, proteins, vitamins, hormones, and other nutrients and factors that support the growth and survival of animal cells in culture (Barnes; Sato, 1980). The typical supplementation proportion of medium with FBS is 10% of the total medium volume, although some cell lines grow satisfactorily with only 5% serum, albeit at slightly reduced growth rates. Eagle's Minimal Essential Medium supplemented with fetal bovine serum is considered a versatile culture medium for mammals, birds, reptiles, amphibians, and fish cells (Lakra; Swaminathan; Joy, 2011) with appropriate adjustment in incubation temperatures. Unlike mammalian cell cultures, fish cells can thrive with infrequent subcultures (every 7-14 days or more) and rarely require changes in growth medium between subcultures (Fryer; Lannan, 1994).

The optimal growth temperature range reflects the donor fish species and its natural environment (Nicholson, 1989). The ease of growing fish cells at a lower temperature compared to mammalian cells may provide cost benefits for cellular fish meat production. Moreover, blending tissue engineering with modern aquaculture techniques presents an attractive opportunity to utilize marine muscle cell culture for *in vitro* fish meat production (Goswami *et al.* 2022).

Cell immortalization and cell stemness

Cells obtained from primary cell culture undergo only a limited number of cell divisions before entering a state of senescence, wherein they experience stable growth arrest. The use of primary culture for cell-based seafood production becomes unsustainable in the long term due to the necessity of maintaining a donor animal as a cell source. To address this issue, the immortalization of target cells for alternative food production is a necessary goal to achieve large-scale production. Cell immortalization disrupts the mechanisms responsible for reaching senescence (Soice; Johnston, 2021).

Embryonic stem cells (ESC) represent a valuable repository of diverse cell morphotypes. Due to their plasticity, stem cells obtained from embryos, classified as totipotent cells, theoretically can differentiate into any cell type of the organism (Rippon; Bishop, 2004). Few researchers have achieved positive results in obtaining somatic cells from ESCs of marine animals under *in vitro* conditions **(Table 2)**. These cells can undergo considerable differentiation, facilitated by supplementing the culture medium with specific factors (Holen; Kausland; Skjærven, 2010).

Species	Embryonic stage	Cellular differentiation	Reference
Sparus aurata (Fish)	Morula	neuron-like and epithelial-like	Vergès-Castillo *et al.* 2021
Gadus morhua (Fish)	Mid-blastula	fibroblast-like and neuronal-like	Holen, Kausland, e Skjærven, 2010
Mytilus trossulus (Mussel)	Trochophore larvae	ciliated cells, muscle cells and neuron-like	Odintsova, Dyachuk, e Nezlin, 2010
Loteolabrax japonicus (Fish)	Blastula	neuron-like and muscle cells	Chen, Sha, e Ye, 2003
Macrobrachium rosenbergii (freshwater shrimp)	Fertilized egg	connective-tissue-like morphology	Sudarshan *et al.* 2024

Table 2: Cells obtained by differentiation of embryonic stem cells delivered from seafood animals

Cell lines are already available from seafood species. Establishing an immortalized lineage from bivalves has been a focal point for numerous researchers due to their social and ecological significance. Unsuccessfully, the only immortalized cell line originating from a mollusk species thus far is the embryonic delivery cell line provided by the freshwater snail *Biomphalaria glabrata* (Wang; Wang, 2019). Various transfection and cell hybridization techniques in crustaceans in shrimp cell culture are being explored as alternatives to establish stable long-term cell lines, showing promising results (Ma; Zeng; Lu, 2017). Only three crustacean cell lines have been established, originating from the shrimp genus *Penaeus*. These cell lines are identified in the *Cellosaurus* database as OKTr-1 (RRID: CVCL_9U40), OKTr-23 (RRID: CVCL_9U41), and PmLyO-Sf9 (RRID: CVCL_A8SX). Both cell lines OKTr-1 and OKTr-23 were described by Tapay *et al.* (1995) and categorized as transformed cells originating from the shrimp lymphoid tissues cell line (Oka). Conversely, the PmLyO-Sf9 cell line was derived from the hybridization of sf9 cells from the insect *Spodoptera frugiperda* with the lymphoid tissue cells of the shrimp *Penaeus monodon* (Anoop *et al.* 2021; Sathyabhama *et al.* 2021).

Most fish cell lines are derived from tissues such as skin, gill, heart, liver, kidney, spleen, swim bladder, brain, etc. Embryos and fins are the most frequently utilized tissue sources for primary culture. After the ovary, the fin is the second most common tissue used for cultivation due to its high regenerative ability (Fryer; Lannan, 1994). An increasing number of marine fish cell lines are available, likely in response to the growing interest in testing viral load, examining water toxicology, and developing vaccines for farmed fish. However, few of these cell lines have been utilized to produce edible fish, apart from a study on producing fish-based proteins for space voyagers on long journeys (Benjaminson; Gilchriest; Lorenz, 2002). Bairoch (2018) listed more than 139,500 cell lines in *Cellulosaurus* database, with about 856 being fish cell lines.

DISCUSSION

Climate change, food supply shocks caused by pandemics, and population growth threaten the traditional food system. Satisfying the demand for meat in the future will be a challenge if we intend to maximize the use of agricultural resources and reduce greenhouse gas production (FAO, 2017). Therefore, disruptive food technologies will be necessary for a more resilient, sustainable, and adequate food system. In this perspective, it is required to consider the factors influencing consumer perceptions of new food technologies, leading to greater acceptance of them (Siegrist; Hartmann, 2020). Informed decisions must be made to achieve scalability, reduce costs, and navigate regulatory challenges effectively. In addition to the core food safety assessments, regulatory considerations may be necessary for other issues such as labeling, consumer preference/acceptance, and ethical or religious aspects of cell-based food products (FAO, 2022).

To discuss the relevant technical issues of cell-based food production, it is important to use clear and consistent terminologies that all the stakeholders can accept. Terminologies and labels are also necessary and direct means of communicating information to consumers (FAO, 2021). Fernandes *et al.* (2019) suggest that, despite a clustering of recurring and highly relevant terms, cultured meat is a subject that spans various areas of knowledge. Nomenclature can significantly impact consumer perception, marketing efforts and relevant regulatory actions such as labeling. While consumer acceptance is critical to the industry's success, the common or usual name chosen to label cell-based products must meet regulatory criteria, not just marketing needs (Hallman *et al.* 2023). A consistent nomenclature is crucial in bringing cultivated protein products to the commercialized market (Malerich; Bryant, 2022). A literature synthesis was conducted on various relevant terminologies by The Food and Agriculture Organization of the United Nations and the World Health Organization (FAO; WHO, 2023). The results showed that while some preferences differ among different sectors, "cell-based food" was less confusing, conveniently overarching, and generally well-accepted by consumers. However, it is essential to note that no term is 100 percent scientifically correct.

Although the terminology is still under discussion, cellular agriculture and cell-based meats have been considered the future of foods. There has been considerable buzz with the launch of next-generation meat alternatives. This field's growing excitement has prompted increasing research, value propositions, business investments, media coverage and discussion. Even so, crucial fundamental research to overcome key technical challenges must be carried out before cell-based meat production can be a reality. Alongside research progress, regulations and standardization of cell-based meat must keep up with the rapid progress in this field (Ong; Choudhury; Naing 2020).

Cultured meat is a promising but early-stage technology with critical technical challenges, including cell source, culture media, mimicking the in vivo myogenesis environment, animal-derived and synthetic materials, and bioprocessing for commercial-scale production (Stephens *et al.* 2018). Thus, one of the technical challenges of this technology is related to the use of animals and products derived from them. As Bhat et al. (2019) mentioned, cell-based meat does not involve slaughtering many animals. However, the initial source of cells and biopsies for starting cell cultures will certainly impact consumers' perceptions and decisions. Legislation regarding cell sourcing and isolation may exist concerning the acquisition of biopsies from live or deceased animals, which could raise animal welfare concerns. Cell bank regulations are also in place in several countries (EMA, 1998; FDA, 2010).

Besides, mass production of cell-based food utilizing traditional cell culture protocols will require hundreds of gallons of fetal bovine serum to produce a few pounds of meat. This implies the continuation of livestock production and an increase in animal exploitation. Due to ethical, environmental, and biological concerns, alternatives to fetal bovine serum or any animal-derived supplement for cell culture are needed. High-volume cell production in industrial bioreactors using serum-free medium is essential for commercial cultured meat manufacturing (Garrison *et al.* 2022). Numerous studies have focused on finding an optimal substitute for FBS. The use of fungi extracts (Benjaminson; Gilchriest; Lorenz, 2002), microalgae extracts (Ng *et al.* 2020), or cyanobacteria (Jeong *et al.* 2021) has shown success in eliminating or significantly reducing the use of FBS in culture. Cell-based food production may also generate new biological or chemical by-products and waste, subject to specific regulations such as environmental legislation. Furthermore, these by-products may be utilized in feed applications if they meet feed safety requirements (FAO, 2022).

On the other hand, one potential future advantage of cell-based meats is the ability to design products with specific nutritional characteristics that are not typically achievable through conventional animal feeding approaches (Faustman *et al.* 2020). Cultivated meat technology can potentially disrupt the food industry; indeed, it is an inevitable reality. This new technology offers an alternative solution to address the environmental, health and ethical issues associated with the demand for meat products. The global market eagerly anticipates biotechnological advancements in the cultivated meat production chain (Santos *et al.* 2023).

A few years ago, cellular agriculture progressed with new research and publications to improve the selection of cell species and cell types to intensify cell-based meat production. Pressing issues such as global warming, environmental instability, and food security have propelled cell-cultured seafood into the spotlight (Rubio *et al.* 2019). The marine environment harbors many bioactive compounds with unique properties, offering significant potential for biotechnological applications (Boziaris, 2014). Seafood production from marine cell cultures represents a novel approach and an exciting opportunity for cellular agriculture. Cell-based seafood holds promising market penetration and sustainability potential, as it can produce meat from species that are challenging to cultivate in traditional aquaculture at competitive prices (Farzad, 2021). Consequently, the emergence of cell-based seafood industries has drawn the attention of aquaculture sectors. However, its market presence remains hypothetical due to consumer acceptance being contingent upon the approval of cell-based meat (Lindfors; Jakobsen, 2022).

Our mini-review of the relevant literature indicates that marine cell and tissue culture research has been largely overlooked. Recent advancements in cellular agriculture underscore the substantial environmental benefits that may result from substituting some industrially raised and processed meat with cultured meat alternatives. There are notable research gaps in marine cell culture that present valuable opportunities for further exploration (Munteanu *et al.* 2021; Rodríguez Escobar *et. al.* 2021). Cell-based meat represents a promising strategy that could offer tools for nutritional enrichment and sustainable seafood generation without the environmental impact associated with traditional methods (Azhar *et al.* 2023).

The future generation of meat substitutes should focus on reducing saturated fat content and using fewer additives (Franca *et al.* 2023). Meanwhile, due to the advances in cultured meat technology worldwide and the slight emphasis that this area has received in no country, we may be missing significant opportunities in this market (GFI, 2022). According to Morais-da-Silva; Villar; Reis (2022), the potential of plant-based and cultivated meat production for creating new and high-skilled jobs has been highlighted. The impact of novel food production systems on employment in conventional meat production may differ for each value chain stage. Technological advancements and investments in cultured meat research suggest that cultured meat will become a mainstream food commodity shortly (Post *et al.* 2020).

CONFLICT OF INTEREST

The authors declare that the research was conducted without any commercial or financial relationships that could potentially create a conflict of interest.

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