Dean&Francis
ISSN 2959-409X

The Scale Effect and Cost Structure Optimization mechanism of cultured meat industrialization

Yuxia Tang[1, *]

[1]College of Food Science and Technology, Hunan agricultural university, Changsha, 410128, China
*Corresponding author: Yuxia0805@outlook.com

Abstract:

The ecological costs of land, water and carbon emissions have long been associated with traditional animal husbandry. Global population growth, in turn, is further increasing the environmental pressures of animal agriculture. Emerging technology of cultured meat has significant potential for application. By promoting the industrialization and commercial application of cultured meat, it helps to protect animal welfare, alleviate food pressure and produce healthy, sustainable meat products in a more environmentally friendly way. This study found that the keys to achieving scale effects and cost control in the cultured meat industry include:1)development of affordable and efficient food-grade culture media by synthesizing growth factors from inexpensive materials such as plant proteins and food industry by-products;2)establishment of a large-scale, controlled and automated production system based on 3D suspension and replenishment batch culture;3) Select the appropriate bioreactor based on cell type, culture size, culture stage, and intended use. In the future, the development of cutting-edge technologies such as cellular immortality and 4D printing may help the cultivated meat industry further expand its production scale and reduce costs, making cultivated meat a more common and sustainable way of meat production. This paper analyses the technological advances and shortcomings in the cost of culture medium, establishment of scale-up system and selection of bioreactors, which are crucial to the three cultured meat industries, with the aim of providing some references for the scale-up production and cost control of cell culture meat.

Keywords: Cultured meat; Cost optimization; Serum-free medium; Scale up; Bioreactor.

1. Introduction

There are many ecological problems that persist in traditional animal husbandry such as the high water consumption, low land use and air pollution. Global population and meat demand are projected to grow by 60% and 70%, respectively, by 2050 [1]. If alternative, more efficient, environmentally friendly and sustainable meat production methods are not developed, the systemic environmental pressures caused by traditional animal husbandry will be exacerbated along with the increase in global demand for meat. For example, increasing the supply of meat requires an expansion of existing farms. However, this is often accompanied by the blind expansion of rangelands or overgrazing, which can easily lead to the reduction of forest areas, the destruction of biodiversity, and the acceleration of soil degradation and environmental pollution. In this context, cultured meat has significant development potential. Cultured meat produced from renewable energy sources, if produced on a large industrial scale, can effectively alleviate the chronic environmental pressures and energy consumption of meat production. The promotion of cultured meat is expected to save 89% of water consumption and 99% of land and reduce greenhouse gas emissions by 96% [2].

However, the current production of cultured meat is expensive due to low yields, high costs and lack of scale. This greatly diminishes its commercial competitiveness. Consequently, producing high value-added products and reducing costs to achieve large-scale production are important strategies to promote the development of cultured meat. The process of cultured meat production can be broadly divided into the following four stages:1)Seed cell isolation and primary proliferation;2)Cell expansion culture using an expansion bioreactor;3)Inoculation of microcarriers and scaffolds for cell differentiation in other types of bioreactors;4) Organisational construction and processing into products. The high cost of cultured meat greatly limits its industrial production and marketing. However, there is a lack of research on techniques related to optimizing the cost of cultured meat. Therefore, based on the process of cultured meat production, this research screened out three key factors affecting the industrial scale and cost of cultured meat, namely the cost of culture medium, the establishment of scale-up system and the selection of bioreactorThe study elaborates on the techniques and methods of optimising the cost structure of large-scale production of cultured meat in these three directions. This study aims to provide a theoretical basis for the development of cultured meat from laboratory research and development to industrial applications and commercialisation.This paper focuses on the three key factors affecting the cost, namely the cost of culture medium, the establishment of scale-up system and the selection of bioreactors, describes the technology to achieve scale-up production of cultured meat and the method to optimize the cost structure, so as to provide a theoretical basis for the over-expansion of cultured meat from laboratory research and development to industrial application and commercial development.

2. Development of Food-grade Culture Media

Media for the pharmaceutical industry are still widely used by the cultured meat industry.Its high selling price greatly increases the cost of cultured meat, which in turn constrains the large-scale production of cultured meat. The cost of cell culture media accounts for 55%-90% of the cost of cell culture meat production [3].Therefore, the development of low-cost food-grade culture media suitable for the safe and efficient production of meat products can effectively reduce the price of cultured meat.Growth factors account for 96% of the total cost of the medium. The use of serum is effective in simulating a stable in vitro growth environment for cells. However, media with only 20% serum can cost as much as $200-500/litre and there is a potential risk of contamination with blood-borne pathogenic contaminants.This implies that the key to the development of food-grade media lies in finding suitable low-cost materials, such as plant proteins or food industry by-products, to replace the source of growth factors in serum media.This not only reduces costs, but also circumvents the risk of cell loss and blood-borne pathogen contamination by unknown parts of the serum complex and improves the feasibility, safety and stability of mass production of cultured meat.In addition, the cost of media supplements, another key barrier to scale-up of cultured meat production, can be effectively reduced with the reduction in the cost of growth factors.

Finding low-cost, efficient and sustainable serum substitutes is key to the development of food-grade culture media. A series of explorations for serum-free media(SFM) laid the foundation for the development of such food-grade media.The cost of Beefy-8 SFM is only $16 per liter, which is much lower than that of serum medium. Moreover, Beefy-9 medium developed by adding recombinant albumin to Beefy-8 can enable bovine muscle stem cells to stably proliferate for 7 generations in vitro, with an average doubling time of less than 40 hours [4].Fur-

ISSN 2959-409X

ther, the use of rapeseed protein isolate to replace recombinant albumin in the development of Beefy-R not only reduces costs but also shortens the average doubling time of bovine muscle stem cells to 26.6 hours [5].In addition, vegetable protein hydrolysates have been shown to be effective serum substitutes.Two studies using plant protein hydrolysates as a substitute for animal proteins have illustrated that they are effective in increasing the proliferation and productivity of animal cells, improving product safety and reducing costs [6,7].In another study on SFM, different food industry by-products such as chicken carcass, cod spine, eggshell membrane, egg white powder, pork plasma, etc. were enzymatically or chemically hydrolyzed to be used as skeletal muscle cell growth factors, and were evaluated using three indexes: metabolic activity, cell proliferation and cytotoxicity effects on muscle cells cultured in vitro. Results showed that the enzymatic products of pork plasma could substitute for serum to effectively improve Skeletal muscle cell metabolic activity by 110% and proliferation rate by 48%, and is safe and non-toxic [8].

While SFM hold significant promise for optimizing the cost of cultured meat production, several existing limitations should be noted: cells grown in serum-free medium are relatively more sensitive to mechanical factor; adaptation to homogeneous SFM can also be affected by cell type; it is also more complex to prepare and preserve compared to traditional synthetic media. Therefore, on the one hand the cells can be modified for low serum domestication using cellular biotechnology; on the other hand, SFM formulations can be optimized for specific cell lines to better mimic the in vivo environment. In addition, the simplified and standardized process of industrial preparation of SFM results in a lower-priced and more stable products, which contribute to the development of inexpensive, food-grade media that can effectively improve cell proliferation efficiency and passaging stability, and are suitable for the current cultured meat industry.

3. Scale-up System Established

3.1 Establishment of a 3D Suspension Training System

Most cultured meat seed cells are adherence-dependent. 2D cell culture is well suited to this characteristic, and it is easy to operate, environmentally controlled, and product homogeneous. However, the approach relies on manual operations on the one hand at a limited scale; on the other hand, it tends to lead to de-differentiation of cells, a phenomenon in which cell lines lose their cellular histological properties. These limit its application to large-scale industrial production. The 3D cell culture model, which mimics the spatial environment of cells in vivo, is a good solution to the problem of cell de-differentiation. The 3D cell culture mode is able to maintain the stability of cell properties and achieve high-density culture, which is more suitable for large-scale production. Domestication of adherent-dependent seeded cells by suspension to adapt to 3D suspension culture enables their more stable, continuous and efficient expansion to meet the standards of industrial-scale high-density cell culture.

A study on suspension continuous culture showed that chicken fibroblasts reached a density of 108 x 106 cells/ml within 15 days, effectively increasing yield to 36% w/v and maintaining good visual and organoleptic analyses [9].Another study on human pluripotent stem cells has also shown that suspension culture can achieve up to 6-fold expansion in a 4-7d culture cycle and more than 10 generations of stable progeny compared to traditional wall culture [10].Consequently, selecting an appropriate culture model to establish 3D cell suspension cultures is crucial for developing large-scale, controllable production processes and automated workflows, thereby enabling the large-scale production and cost optimization of cultured meat.

However, suspension domestication works by using mechanical shear to inhibit cellular shear dependence, making it difficult to establish current suspension culture systems for highly shear-sensitive cells such as muscle stem cells.A review of engineering principles for optimal suspension culture conditions proved that suspension culture in zero headspace culture vessels such as solid rotating couette streams, rotating wall vessels, etc., and the selection of microcarrier beads and media with densities as close as possible to each other, minimized mechanical damage and optimized the differentiation of cultured cells [11].In addition, low-shear bioreactors can also be preferred for cell characteristics, and all physiological and biochemical indicators and metabolic patterns of the cells can be dynamically monitored in order to establish a suitable, efficient and scaled-up suspension culture system.

3.2 Cell Culture Model Optimization

Critical factors in cell culture include the provision of adequate nutrients for efficient cell growth and metabolism, timely regulation of catabolic metabolites, and contamination prevention and control. Common modes of cell culture operation include batch culture, continuous culture, perfusion culture, and replenishment batch culture. Batch cultures are easy to operate but are not conducive to cell

passaging and are therefore not suitable for large-scale production of cultured meat. Continuous cultures are also rarely used in large industrial production lines due to contamination and genetic stability risks. Although perfusion culture has significant advantages in increasing cell density and yield and can be used in conjunction with mechanical cell immobilization in the cell differentiation stage, it has the disadvantages of high cost, complexity and risk of contamination.

The relative simplicity, cost and yield advantages of replenishment batch culture make it the most widely used in the industry today.For example, a study of batch and replenished batch cell cultures calculated that replenished batch cultures reduced media consumption by 70% [12]. Another study on the application of batch, supplemented batch and continuous cultures to fermentation also revealed that supplemented batch cultures resulted in higher product concentrations and higher yields than the other cultures [13].A study evaluating the extent of cell culture scale-up demonstrated, through a techno-economic analysis showed that the total cost of replenishment batch culture ($37/Kg) was lower than that of perfusion culture ($51/Kg) under the same conditions and that culture conditions were more homogeneous, and that a replenishment batch process using low-cost hydrolysate media might result in a lower price of cultured meat than $25/Kg, improving the affordability of cultured meat [14]. Consequently, the development of inexpensive medium supplements and the application of the supplements to batch cultures can help to reduce the cost of cultured meat production, expand the scale of industrial production, and significantly increase the competitiveness of cultured meat in the market.

4. Bioreactor Selection

Key factors to consider for bioreactors when scaling up cultured meat production include: reactor geometry, agitator shape and speed, mass transfer efficiency, dissolved oxygen levels, heat dissipation and avoidance of inhomogeneity. Therefore, the large-scale production of cultured meat requires the selection of an appropriate bioreactor based on cell type, size and intended use. Bioreactors currently in common use for cultured meat include: Stirred Tank Bioreactor(STR), Airlift Bioreactor(ABR), Packed-Bed Bioreactor(PBR), Oscilllatory Baffled Bioreactor(OBR), Hollow Fibre Bioreactor(HFR).Their advantages, disadvantages, applicable cells and products are shown in Table 1.

Table 1. Comparison of Commonly Used Bioreactors in cultivated-meat Industry

Type	Advantages	Disadvantages	Applicable Cells	Applicable products
STR	Good mixing;High oxygen transfer efficiency;High scalablility and widely used	High shear stress damages fragile cells;high energy consumption	suspension cells	Ground meat products; Paste products
ABR	Low shear stress good for fragile cells;Energy-efficient	Lower mixing efficiency than STR;Limited scalability for high-density cultures	vulnerable cell	High value-added products
PBR	High surface area savings on costs of culture media and operations;High space utilisation;Suitable for continuous production	Difficulty in scaling up training;Uneven nutrient logistics	adherent cell	structured meat
OBR	Low shear stress;Easy to build;Simple to operate;Disposable;Suitable for pre-optimisation	Limited scalability;Not suitable for high density culture	Small- and medium-scale cell culture	Small batch test products
HFR	Low shear stress;Practical at the stage of cell differentiation	Cell density and high microfibres can limit cell harvesting efficiency	myofibroblast;fibroblast	structured meat

Cultured meat cell culture is mainly divided into two stages: expansion and differentiation. The amplification phase aims to obtain the highest possible cell density. Therefore, it is suitable for cell immobilization by microcarriers and culture in dynamic hybrid bioreactors such as STR and RBR. Static bioreactors, on the other hand, are more advantageous during the differentiation phase, as the smaller shear stress is less able to better simulate the actual conditions of myogenesis. For instance, HFR can mimic the role of blood vessels for nutrient supply and metabolite

ISSN 2959-409X

removal [15].The PBR has a cell-immobilized packed bed structure with slow medium circulation, and high animal cell densities can be achieved through the application of the perfusion mode of operation. The use of PBR for muscle cell proliferation or the final step of seed culture procedures may result in meat products with good texture. To summarize, the selection of a suitable bioreactor based on cell type, culture scale, culture stage and intended use could help to establish a scaled-up, automated production process and optimise the cost and efficiency of cultured meat production.

5. Conclusion

Cultured meat has great potential for application: relieving pressure on land and water resources, air pollution and biodiversity destruction caused by animal husbandry reflects ecological values; it also helps to alleviate problems such as the misuse of antibiotics in the aquaculture industry and achieve efficient, safe and sustainable production of meat products; the ability to produce meat products that cannot be raised by traditional livestock farming, or more economically produce high value-added products, demonstrates commercial value. However, currently cultured meat is limited by industrial scale and production costs, making commercial application and promotion difficult. Based on the cultured meat production process, key aspects of reducing its cost include: searching for cheap and suitable materials such as plant proteins and food industry by-products for the synthesis of growth factors, which can be applied to the development of low-cost and high-efficiency food-grade culture media; establishment of a large-scale, controlled, automated production system based on 3D suspension and replenishment batch culture; select the appropriate bioreactor based on cell type, culture size, stage of culture, and intended use.

In the future, cultured meat may be able to increase the scale of production and commercial competitiveness in these ways: explore safe and efficient cell immortalization technology to build a seed bank of good cells; integration of, for example, computational fluid dynamics, spatial and temporal multi-scale coupling simulation technology to build a multi-level dynamic intelligent model, to achieve precise control of the production process and intelligent production; Combining emerging technologies such as 4D printing to solve existing challenges such as cultivating meat vascular networks, further enhancing meat quality to produce high value-added products; Continuously promote the improvement of the regulatory system, and increase the popularization of science to enhance consumer acceptance. The development and dissemination of cultured meat technology may facilitate the production and consumption of more economical, healthy and sustainable meat products in a more environmentally friendly way, reducing the pressure on meat demand that accompanies population growth.

References

[1] Gerber P J, Steinfeld H, Henderson B, et al. Tackling climate change through livestock: a global assessment of emissions and mitigation opportunities[M]. 2013.

[2] Gomez Romero, S., & Boyle, N. (2023). Systems biology and metabolic modeling for cultivated meat: A promising approach for cell culture media optimization and cost reduction. Comprehensive Reviews in Food Science and Food Safety, 22(4), 3422-3443.

[3] Reiss J, Robertson S, Suzuki M. Cell Sources for Cultivated Meat: Applications and Considerations throughout the Production Workflow. International Journal of Molecular Sciences. 2021; 22(14):7513.

[4] Stout A J, Mirliani A B, Rittenberg M L, et al. Simple and effective serum-free medium for sustained expansion of bovine satellite cells for cell cultured meat[J]. Communications Biology, 2022, 5(1): 466.

[5] Stout A J, Rittenberg M L, Shub M, et al. A Beefy-R culture medium: Replacing albumin with rapeseed protein isolates[J]. Biomaterials, 2023, 296: 122092.

[6] Burteau C C, Verhoeye F R, Molsl J F, et al. Fortification of a protein-free cell culture medium with plant peptones improves cultivation and productivity of an interferon-γ-producing CHO cell line[J]. In Vitro Cellular & Developmental Biology-Animal, 2003, 39(7): 291-296.

[7] George, F., Kerschen, D., Van Nuffel, A., Rees, J. F., & Donnay, I. (2009). Plant protein hydrolysates (plant peptones) as substitutes for animal proteins in embryo culture medium. Reproduction, Fertility and Development, 21(4), 587-598.

[8] Andreassen RC, Pedersen ME, Kristoffersen KA, Beate Rønning S. Screening of by-products from the food industry as growth promoting agents in serum-free media for skeletal muscle cell culture. Food Funct. 2020 Mar 26;11(3):2477-2488. doi: 10.1039/c9fo02690h. PMID: 32134068.

[9] Pasitka L, Cohen M, Ehrlich A, et al. Spontaneous immortalization of chicken fibroblasts generates stable, high-yield cell lines for serum-free production of cultured meat[J]. Nature Food, 2023, 4(1): 35-50.

[10] Zweigerdt R, Olmer R, Singh H, et al. Scalable expansion of human pluripotent stem cells in suspension culture[J]. Nature protocols, 2011, 6(5): 689-700.

[11] Hammond T G, Hammond J M. Optimized suspension

culture: the rotating-wall vessel[J]. American Journal of Physiology-Renal Physiology, 2001, 281(1): F12-F25.
[12] Lam A T L, Li J, Toh J P W, et al. Biodegradable poly-ε-caprolactone microcarriers for efficient production of human mesenchymal stromal cells and secreted cytokines in batch and fed-batch bioreactors[J]. Cytotherapy, 2017, 19(3): 419-432.
[13] Paulova L, Chmelik J, Branska B, et al. Comparison of lactic acid production by L. casei in batch, fed-batch and continuous cultivation, testing the use of feather hydrolysate as a complex nitrogen source[J]. Brazilian Archives of Biology and Technology, 2020, 63: e20190151.
[14] Humbird D. Scale-up economics for cultured meat[J]. Biotechnology and Bioengineering, 2021, 118(8): 3239-3250.
[15] Pajčin I, Knežić T, Savic Azoulay I, et al. Bioengineering outlook on cultivated meat production[J]. Micromachines, 2022, 13(3): 402.