PROTEIN HYDROLYSATES APPLIED TO CULTURED MEAT PRODUCTION

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ABSTRACT

Population growth is a concern due to the increasing demand for high-nutritional-value foods, such as meat. Due to environmental issues, researchers and industries have accelerated research into alternative proteins. Among these, cultivated meat stands out, developed through multidisciplinary technological bioprocesses. Cultured meat is produced using fetal bovine serum, a nutrient-rich culture medium supplement, although this is responsible for the high cost of the process and raises ethical concerns due to its animal-origin. Considering cost and environmental impact factors, plant protein hydrolysates are considered a viable alternative for producing cultivated meat. The plant protein hydrolysates characterized by researchers present free amino acids, peptides, vitamins, proteins, and growth factors of great interest for cell culture. This review presents the technical factors related to the potential application of non-animal protein hydrolysates as substitutes for fetal bovine serum components. Continuous research is essential and promising for developing non-animal culture media for producing cultivated meat.

Keywords: culture medium. alternative protein. protein hydrolysates.

1 INTRODUCTION

The population surge is intricately linked to the demand for food, primarily of animal origin. Large-scale conventional meat production is associated with environmental degradation, gas production that impacts in the global warming, and animal suffering. In this context, researchers, companies, and shareholders are urgently exploring and investigating alternative proteins sources. Cultured meat, an alternative protein developed through a series of technological processes, including tissue engineering and biomaterial sciences[1], emerges as a potential solution to this pressing issue. This product consists of bioengineered materials derived from animal cell biomass obtained through cell culture processes after *in vitro* expansion and muscle cells differentiation.

The culture medium is a pivotal element in the production of cultured meat, directly influencing the cost of the production process[2]. The primary supplement for cell culture, considered universal, is Fetal Bovine Serum (FBS). It contains a high concentration of growth factors and hormones that facilitate the growth of most cell types. FBS, despite its richness in nutrients and hormones that stimulate cell proliferation and growth, has its drawbacks. The principles that underpin the adoption of alternative proteins, such as environmental consciousness and the avoidance of animal suffering, are compromised using FBS. In addition to the animal-origin source of FBS, batch-to-batch variability undermines the efficiency and scalability of the process due to the risk of microbiological contamination, poor reproducibility, and high costs[2]. In this context, the potential of plant-derived protein hydrolysates as a promising culture medium supplement for the cultured meat market becomes even more significant.

Protein hydrolysates derived from vegetables offer a complementary array of proteins, peptides, amino acids, lipids, and vitamins[1]. The author underscores their positive attributes, such as antioxidant, antibacterial, and immunomodulatory properties, which are conducive to cell growth and enhance the nutritional and sensory profiles of cultured meat. This review will delve into the viability of protein hydrolysates as a viable substitute for fetal bovine serum, shedding light on their potential in the cultured meat industry.

2 CULTURED MEAT

The development of cultured meat was promoted by concerns linked to population growth, environmental impact (livestock farming, land use, greenhouse gas emissions), reduction of animal suffering (living conditions and slaughter), and consequences for human health[3]. Cultured meat is composed of cells from animal muscle tissue and can reproduce the sensorial and nutritional profile of traditional meat[4].

The term cultured meat is also known as synthetic meat, *in vitro* meat, cell-based meat, clean meat, or laboratory-grown meat and combines traditional cell culture and biofabrication techniques with the aim of forming arrangements similar to animal meat[567]. The four stages of cultured meat production are: 1) obtaining seed cells, 2) multiplication of seed cells, 3) differentiation of cells into myofibers, adipocytes, among other muscle cells, 4) processing of cells into meat products[8]. The process is complex, and a series of control points are required for each stage due to consumer food safety issues.

In short, an animal's muscle biopsy is initially performed to isolate stem cells and subsequently differentiate into different cell types, such as fibroblasts, muscle and fat cells[3] produced in a sterile laboratory[9]. The process, despite being complex, has seen accelerated development since the first cultured meat CS (laboratory burger) was produced and tasted on television in 2013[10].

Another important point for the development of cultured meat is the culture medium (nutrients) in which the cells multiply under controlled conditions and develop cell fibers[11]. Stem cells for cultured meat production are considered the most interesting option as they could generate and specify different lineages[3].

The researcher mentioned above also informs that adult stem cells (limited differentiation potential) and pluripotent stem cells (wider differentiation capacity) are considered two sources for cultured meat cultivation[3]. In addition to the topics covered, the scientific community, media and stakeholders question the advantages/disadvantages, legislation, market and nutritional factors of the cultivated meat, as it represents a breakthrough regarding the food habits.

3 ISSUES RELATED TO THE USE OF FETAL BOVINE SERUM

Fetal bovine serum (FBS) is widely used as a supplement for animal cell culture due to its rich combination of nutrients, hormones, and enzymes, which promote cell proliferation and differentiation[8]. This supplement represents approximately 80% of the costs in the cultured meat industry and is, therefore, one of the main factors contributing to the high cost of production. Additionally, FBS is derived from animal sources, which goes against one of the fundamental goals of cultured meat: production without animal suffering. Discussions surrounding cultured meat production also address questions about the use of bioreactors and associated energy consumption, especially in comparison to conventional meat production[1].

Some serum-free media and additives are sold on the market and have shown positive results for cultivating mesenchymal, hematopoietic, and neural stem cells[8]. Developing a low-cost cultivation medium that does not come from animals is essential for this market as, in general, consumers of alternative proteins consider factors such as price and environmental concerns. Dr. Marcus Johannes Post's team compared three serum-free commercial media and two serum substitutes with growth sources to multiply bovine myoblasts, resulting in an increasing in cell number for six days of culture, while in the serum-containing medium, the result was negative[8].

The demand for nutrients differs between cell lines as they have different metabolic demands[18]. Therefore, it is essential to note that serum-free media may reduce the differentiation and cell growth compared to fetal bovine serum[1]. The authors mentioned above state that the commercialized compounds of amino acids and proteins are not produced in quantity to meet the production of the food industry. Culture media free of fetal bovine serum need to be studied, formulated, and applied to promote food security and productivity in the cultured meat industry.

4 POTENTIAL PROTEIN HYDROLYSATES

Hydrolysates proteins are produced through hydrolysis by acidic, alkaline, enzyme, and fermentation processes and can come from both vegetable and animal proteins[2]. Enzymatic hydrolysis is most used in the food industry because other methods leave organic and chemical residues during the process[12]. The acid/base hydrolysis process results in a complex compound of oligosaccharides, proteins, free amino acids, and carbohydrates[13].

Although fetal bovine serum is a source of nutrients and contributes to cell culture, animal component-free media[2], such as a combination of recombinant proteins and protein hydrolysates, are considered promising media for partial replacement of cell culture media for the cultured meat industry[13]. Vegetable residues from the food industry applied as hydrolysates are materials rich in essential nutrients[1] that are discarded and incinerated, and some are destined for cattle feed[14].

Hydrolysates from vegetables, acting as a successor to albumin, guarantee cellular resistance to shear stress and contribute to robustness[1]. In the study[1] mentioned that soy hydrolysates improve cell growth and have multiple bioactivities and compounds (citrulline and ornithine) that are interesting for the food and pharmaceutical industries. Linseed and rice hydrolysates have low molecular weight (1-3kDa) and a better antioxidant effect than peptides larger than three kDa[2]. Rice and wheat protein hydrolysates contain peptides that interact with cell surface receptors, promoting cell growth and protein biosynthesis[1]. Chickpea protein hydrolysate is an adequate substitute for fetal bovine serum (FBS) in suspension cell cultures; however, no increase in cell growth was observed in monolayers[1]. In contrast, in Chinese hamster ovary culture, flaxseed meal protein did not show growth rates or productivity[1]. Finally, cottonseed meal showed significant increases in cell density and recombinant protein production by Chinese hamster ovary cultures[1]. As previously mentioned, several nutrients contribute to protein hydrolysates being considered promising as a culture medium for cultured meat.

5 CONCLUSION

Cultured meat is a promising viable alternative for developing foods like traditional meat in a sustainable manner using cellular agriculture technology[7]. The reduction in animal husbandry for slaughter and microbiological risk (increased resistance to antibiotics used in veterinary medicine or growth stimulants)[15] is a positive point, as is the reduction in environmental problems (greenhouse gases) that may influence consumers to consume cultured meat.

The high cost of FBS which accounts for a significant proportion of production expenditure and scalability limitations has spurred research in the field to develop alternative mediums rich in nutrients, free amino acids, vitamins, and peptides. This review focuses on protein hydrolysates, non-animal substitutes for FBS. Among the various options available, it becomes evident that protein hydrolysates are viable inputs to produce cultured meat, underscoring the importance of this research.

Studies focused on the characterization of vegetable protein hydrolysates can provide a detailed understanding of their components and functionalities, which are essential for developing culture media free of fetal bovine serum. An in-depth analysis of these aspects contributes to reducing production costs and make protein hydrolysates from industrial waste a sustainable alternative to replace the supplementation conventionally used for these culture media.

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