The Application and Safety of Novel Additives in Artificial Meat

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Abstract. As an emerging food, artificial meat is gradually taking over the catering market. The application and safety of its new additives in food have drawn much attention. The application demand for new additives in both plant-based meat and cell-cultured meat is increasingly widespread, but it faces challenges in the field of food safety. This article reviews the application and potential risks of two core types of additives in artificial meat. The research found that some genetically modified additives, flavor enhancers, cell scaffold materials, and culture medium components, etc., can improve the quality and sensory characteristics of artificial meat within a reasonable usage range, but toxicological and other experiments have shown their safety risks. This article aims to provide theoretical support for the standardization, safety assessment, and policy formulation of the artificial meat industry, and to promote its safe and sustainable development in the artificial meat market.

Keywords: Novel additive, Cell-cultured meat, Plant-based meat, Genetic engineering.

1. Introduction

With the global population experiencing a sharp increase, the market is facing a shortage of meat supply and a global protein supply crisis. According to data from the World Health Organization, it is projected that by 2050, the meat industry will need to increase production by 50% - 73% to meet the growing demand of the population. By then, the amount of meat consumed by humans will be 70% [1-2] more than that in 2005.While bringing about environmental pressure and animal welfare issues, excessive consumption of traditional high-cholesterol meats may cause cardiovascular and other diseases, threatening human health. According to statistics, 22% of foodborne pathogens originate from meat products [3], and it is predicted that by 2050, bacterial antimicrobial resistance will cause 10 million deaths each year. As a sustainable alternative to traditional livestock farming, the development of the artificial meat industry has become an inevitable trend in the global food sector. Plant-based meats (such as Beyond Meat, Impossible Foods) and cell-cultured meats (such as Good Meat, UPSIDE Foods) are gradually entering the consumer market by mimicking animal meat. According to a report released by Zhongyan Network, the global market size of artificial meat is expected to experience explosive growth by 2030, with the market expected to exceed 25 billion US dollars. North America and Europe are the core markets. Taste and appearance are the key factors for people to choose food. To fully achieve realistic color, texture and flavor, additives are indispensable. Traditional additives have gradually failed to meet the demands of humans for both taste and health and environmental protection. Currently, there is no global unified definition for new additives, but most consider them to have innovations and advantages in modern new technologies, processing techniques, application scenarios, targeted functional design, and clean production. They refer to artificial synthetic or bio-engineered components that have been developed or newly approved in recent years. For plant-based meat, it is necessary to add genetically modified soybean hemoglobin (LegH) and other pigments to enhance the "meaty" texture; for cell-cultured meat, it needs to adhere to biological scaffold materials and growth factors to support cell proliferation, and subsequently, its food safety has become a research focus.

Even though new additives have promoted technological progress and consumers' acceptance of products, there are still some potential health and ecological risks, as well as market scale-up challenges, which deserve further in-depth discussion. This article reviews the progress in the application and safety assessment of artificial meat additives, providing a theoretical basis for food safety research and consumer protection, with the aim of promoting the safe and steady development of the artificial meat field.

2. The Application and Necessity of the New Type of Additive

2.1. The limitations of traditional additives

Many traditional additives are derived from non-natural raw materials and inedible synthetic materials, lacking functionality and palatability, and thus cannot fully simulate real meat. As a result, they are severely restricted in the production of artificial meat.

2.1.1. Limitations in texture and taste

The traditional chemical preservative potassium sorbate fails to meet the biocompatibility requirements of cell-cultured meat. The traditional pigment nitrite can only color but cannot simulate the flavor. Traditional starch-based adhesives cannot maintain the structure of plant-based meat at high temperatures. Excessive addition will also lead to a stiff texture and reduce the juiciness of the product [4]. Traditional tissue engineering scaffolds mostly use inedible synthetic materials, such as polycaprolactone.

2.1.2. Ecological risk

Traditional additives are overly dependent on animal-derived organisms. According to statistics, 5.6 kg of CO_2 is produced per kilogram of gelatin, mainly from livestock farming and high-temperature processing [5]. Traditional culture media use fetal bovine serum as the serum carrier (FBS), accounting for 60% of the total cost of the culture medium. Not only is the cost high, but it also requires slaughtering pregnant cows, which damages biodiversity. Traditional carrageenan relies on seaweed fishing. According to data from the FAO and the World-Wide Fund for Nature, about 90% of the raw materials for carrageenan in the world are obtained from wild red algae, mainly from the Philippines and Indonesia. The fishing volume far exceeds the natural regeneration rate, resulting in the reduction of red algae beds and the decline of fish populations in Southeast Asian waters, threatening coral reef ecology, and bottom disturbance will ultimately lead to the destruction of the marine ecosystem.

2.2. Technology and Application of New Additives in Plant-based Meat Products

Plant-based meat is prepared from plant proteins through techniques such as high-moisture extrusion, tissue modification by shear force, heat processing, electrospinning, etc., to produce products with meat-like texture similar to that of livestock, poultry, and aquatic products, in order to simulate the fiber structure and flavor and texture of animal meat. New-generation additives, which are derived from natural extracts and utilize modern processing techniques and biotechnology means, also exert their effects in plant-based meat as "new-generation" clean additives.

2.2.1. Reinforced adhesive

To avoid the "bean pulp-like" loose texture of plant-based meat, a binder is needed to reconstruct the texture. Novel binders can be derived from the combination of single materials. Based on the pea protein-κ-carrageenan complex subjected to high humidity extrusion, a the irreversible gel cross-linking system can be formed, simulating the meat fiber structure, achieving the "pseudo-meat-like" gel-melting behavior of plant-based meat upon heating, enhancing the stability of plant-based steak, roast meat, etc. under high temperatures, and achieving the texture characteristics that cannot be reached by traditional single components [6]. It improves the chewing strength, tearing sensation and juice release of plant-based meat. Microencapsulated fats formed by coconut oil, sunflower seed oil and emulsifiers (such as lecithin) imitate the melting characteristics of animal fats, which is helpful for the production of plant-based products such as five-variety pork, bacon, etc. that require fat textures.

2.2.2. Developer

The red beet extract containing natural beet red pigment or betacyanin is prone to degradation during cooking and can be used as a heat-labile red colorant to simulate the color changes of real meat during cooking; algal-based pigments (such as phycocyanin) are natural blue pigments extracted from spirulina and can also replace artificial synthetic pigments for color simulation in plant-based seafood products (such as vegan fish fillets) [7]. In the market of plant-based meat production, Beyond Meat uses beet juice and apple extract to imitate the red color of meat, and Impossible Food uses soy hemoglobin to simulate the heme in meat.

2.2.3. Enzymatic digestion, fermentation and transgenic technology

The application of technological means is conducive to the production of different meat foods. Through the targeted enzymatic hydrolysis technology, generating small molecule peptide segments of pea protein hydrolyzed peptides can improve the solubility and texture of plant protein, and reduce allergenicity, and can be used for the production of high-protein plant meat patties, vegetarian chicken pieces, etc. [8]; through microbial fermentation production of fibrous fungal protein, it provides similar animal muscle texture, such as plant-based chicken pieces, meat slices, etc. (like the core technology of Quorn) [9]; through microbial fermentation technology, specific strains can convert soybean residue into "smoky flavor substances" for the simulation of bacon aroma in plant-based meat. The "soybean-like flavor" of plant protein and the lack of heme pigment leading to pale color are long-term pain points in the industry. Yang et al. used gene editing engineering technology to modify the genes of yeast cells, after fermentation, soybean hemoglobin was produced [10], its properties are close to myoglobin, and during heating, soybean hemoglobin denatures, its structure unfolds and exposes heme, heme catalyzes the reaction of plant-based biomolecules in the ingredients to generate "meat aroma" molecules, thereby generating flavors and odors similar to heated meat, suitable for making plant-based meat foods such as hamburger meat patties and sausages that require a "bleeding" effect.

2.3. Technology and Application of Novel Additives in Cell-Cultured Meat

Cellular cultured meat technology, by utilizing animal stem cells or muscle cells to proliferate and differentiate in a sterile environment [11], enables large-scale in vitro cultivation of animal cells and tissues, ultimately forming meat products similar to traditional meat in structure and nutritional composition. Key components include cell culture media, serum, scaffold materials, etc. Its production process highly relies on cutting-edge biotechnological engineering, with extremely high costs and facing numerous challenges, such as difficulty in large-scale production and commercialization. New additives are the architects of cells, from the laboratory to the dining table, the birth of a piece of cellular cultured meat is not only a revolution in biotechnology, but also a "genetic modification" of food additives. This technological wave that overturns traditional animal husbandry conceals an unheard-of transformation - cellular cultured meat is reshaping the definition and boundaries of food additives. Current research focuses on serum-free culture media, 3D bioprinting biomimetic structures, and nano-encapsulation technologies, achieving performance optimization through multi-scale regulation, and transforming the cellular cultured meat industry towards clean production and precise functional design.

2.3.1. Cellscaffolding materials

Typical cell scaffolds include 3D printing and Biphasic hydrogels, etc. 3D printing scaffolds are novel edible scaffold materials with good biocompatibility, mainly used to simulate extracellular matrix (ECM), providing three-dimensional support while supporting cell adhesion, proliferation and differentiation. Their porous structure can deliver oxygen and nutrients to cells and guide their growth. Through material design, the texture can be optimized. A pure plant-based 3D printing ink based on gluten albumin, combined with electrostatic field electroosmotic jetting technology (EHDP), has fabricated ultra-fine protein fiber scaffolds that can support the adhesion and differentiation of muscle stem cells. Some low-cost biodegradable scaffolds, such as cellulose, chitosan, plant-based polysaccharide scaffolds like cellulose and chitosan, and edible fungal mycelium scaffolds, support cell layering and can enhance the reuse of agricultural waste; Double-network hydrogels are also functional scaffolds, belonging to polysaccharide-protein complexes, such as sodium alginate and plant protein or gelatin composites. Traditional hydrogels are difficult to achieve precise release and aroma retention in staged or condition-triggered release due to their single-phase state. However, biphasic hydrogels can trigger the release of bound flavor compounds when cooked at about 150 degrees Celsius, simulating the aroma of real meat, and the sustained-release property can prolong the retention time of flavor substances. Their biphasic structure simultaneously provides flexible support (simulating muscle tissue) and rigid network (maintaining shape), making them more suitable for the growth requirements of animal stem cells, and can be used to manufacture meat with real fat, muscle texture and nutritional characteristics [12].

2.3.2. Serum-free culture medium

Serum-free culture medium can enhance cell proliferation efficiency by using plant-derived growth factors (such as recombinant insulin-like proteins) and synthetic small molecule inducers (such as ROCK inhibitor Y-27632), and its components can replace fetal bovine serum (FBS), avoiding ethical controversies and pathogen risks. Through synthetic biology technology, more than 500 kinds of nutrients in serum have been precisely replicated, such as recombinant human transferrin replacing animal-derived iron carriers, plant-derived growth factors simulating the proliferative effects of EGF and FGF, and nano-sized liposomes encapsulating vitamins and trace elements, etc.

2.3.3. Sensory enhancers

Additives can be used as sensory enhancers, cracking the taste code of real meat and enhancing nutrition. Hemoglobin protein engineering utilizes Pichia pastoris to express plant-derived hemoglobin; Maillard reaction accelerators, nano-scale ferrous phosphate catalyzes the reaction between amino acids and reducing sugars; texture regulation enzymes, which are directed to hydrolyze collagen to enhance tenderness. In addition, some additives can precisely regulate differentiation pathways and reduce the generation of non-target cells. For instance, natural plant extract curcumin inducers increase the juiciness of meat by promoting the differentiation of stem cells into adipocytes.

3. Safety Assessment of New Additives Used in Artificial Meat

3.1. Human Health and Safety

3.1.1. Plant-based meat

The genetically engineered soybean hemoglobin is produced by yeast cells through genetic modification. Based on the research on the allergenicity of transgenic soybeans, it was found that during the transgenic process, proteins may be introduced or changed in structure, resulting in the generation of new allergens, posing a risk of sensitization. People with allergic constitutions are more sensitive to the new hemoglobin. According to the announcement made by EFSA in 2022, "LegH contains a 34 kDa protein, which may trigger cross-reactions in soybean allergy patients". Soybean hemoglobin contains heme iron, and in vitro experiments have shown that high-dose heme iron can induce DNA damage in colon cells. Iron overload may promote the generation of free radicals in the intestine and cause oxidative damage [13], posing a threat to patients with hemoglobin deposition syndrome. Studies have shown that the iron transport protein in soybean root nodules is inhibited by inorganic nitrogen, which may interfere with the iron metabolism balance, and transgenic hemoglobin may exacerbate this process. However, based on molecular characteristic data and bioinformatics analysis data, the EFSA GMO expert group did not find any safety issues regarding toxicity and allergenicity of this soybean hemoglobin. They believe that soybean hemoglobin will not cause safety problems under the predetermined conditions [14].

The main active components in the antioxidant rosemary extract include carnosic acid and carnosol, etc. Among them, carnosic acid may generate quinone intermediates during human metabolism and form adducts with DNA, thereby increasing the risk of gene mutations. In addition, the rosemary extract may exhibit pro-oxidative effects in food systems containing iron ions, leading to an increase in lipid peroxidation products (such as malondialdehyde) [15], posing potential risks for patients with iron deficiency anemia or pregnant women. Moreover, during extrusion and high-temperature processing, it may decompose and generate polycyclic aromatic hydrocarbons (PAHs) such as benzo [a]pyrene, whose toxicity has been classified as a Group 2 carcinogen by the EFSA in the EU assessment. According to the EFSA safety assessment report, the rosemary extract (E 392) should be consumed at the recommended dose (< 400 mg/kg food).

3.1.2. Cultured meat from cells

There may be risks of residual harmful substances in both the scaffold materials and the components of the culture medium. Scaffold materials can be classified into synthetic polymer scaffolds (such as PLA, PCL) and natural polymer scaffolds (such as collagen, alginate). The lactate monomers produced by the degradation of polylactic acid (PLA) accumulate in the body and may lead to metabolic acidosis; the degradation products of polycaprolactone (PCL) may interfere with lipid metabolism; during the extraction of alginate, heavy metals such as aluminum and lead may be retained, and long-term intake may damage the nervous system. The growth factors and antibiotic substitutes in the culture medium components have potential toxicity. For example, the residual of IGF-1 and FGF-2 may interfere with human metabolism and increase the risk of breast cancer [16]; The risk assessment by the European Union shows that the detection rate of residual antibiotics (such as gentamicin) in cell-cultured meat is 5%, and they may accumulate through the food chain.

Furthermore, studies have shown that the use of 3D printing auxiliaries may be accompanied by the release of two types of risk substances: nanocellulose (Cellulose Nanofibrils, CNFs) and microplastics (Microplastics, MPs). Among them, CNFs can induce local inflammatory responses by penetrating the intestinal mucosal barrier; the nanoparticles cultured by magnetic levitation may accumulate in the liver, causing oxidative stress damage to liver tissue. On the other hand, the microplastics particles released from material wear during 3D printing process may interfere with the intestinal microbiota and disrupt the microbial homeostasis.

3.2. Environmental and Ecological Safety

3.2.1. Risk of gene pollution and antibiotic residues

Genetic pollution constitutes a new type of ecological threat, encompassing microbial escape and gene dissemination. Recombinant microorganisms that remain partially alive in production additives may enter the environment through wastewater discharge and undergo horizontal gene transfer with wild strains, resulting in drug resistance or disruption of ecological balance. For instance, the heterologous genes carried by transgenic yeast may integrate into the natural microbial community, affecting the microbial diversity in soil or water bodies. Beyond Meat's genetically modified yeast-produced soybean hemoglobin gene may be transferred to wild soybeans or other plants through pollen or microbial vectors, thereby disrupting ecological balance. If the fermentation strains escape into the natural environment, they may influence the wild yeast population through horizontal gene transfer. Experiments have shown that transgenic microorganisms have a survival rate of up to 30% in simulated natural environments, significantly higher than that of non-transgenic strains. Cell culture requires long-term use of antibiotics, such as penicillin and streptomycin, to inhibit microbial contamination. Residual antibiotics, through wastewater discharge, may promote the evolution of drug-resistant bacteria in the environment. In 2022, research found that the antibiotic concentration in the wastewater from meat cultivation factories could reach 5-8 times the safe standard for surface water (Figure 1).

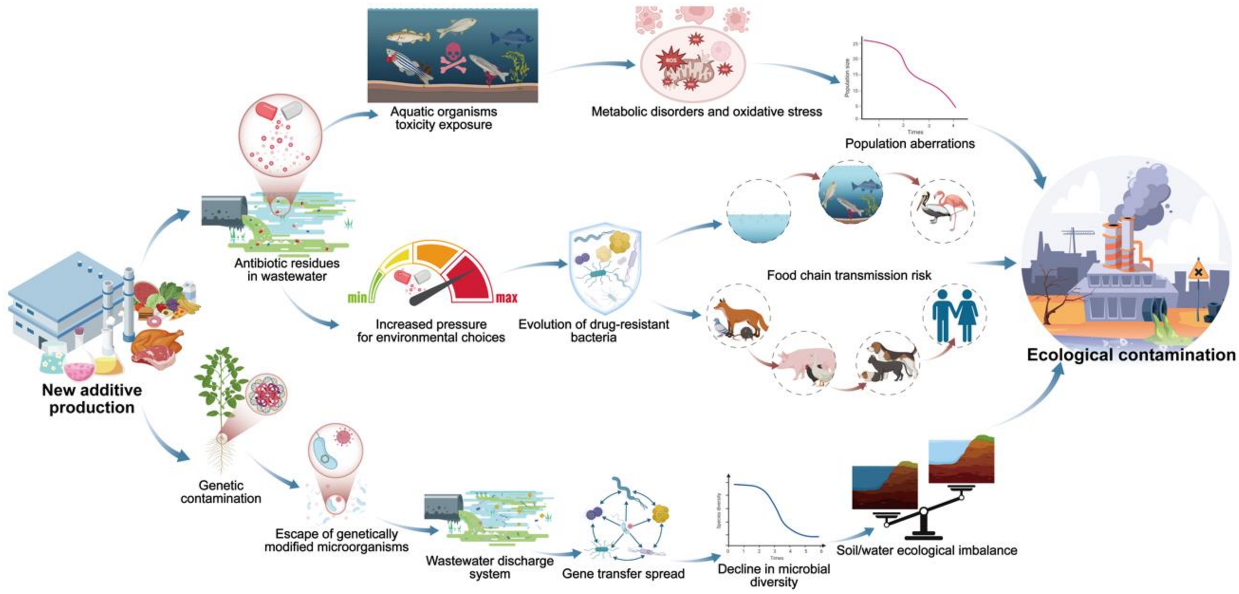

Figure 1. Spread of drug-resistant bacteria and genes among different One Health sectors

3.2.2. Pollution occurring during production process

During the production process, microplastic pollution, chemical waste discharge and energy consumption along with carbon emissions may be involved. Among them, microplastic pollution is formed by the microcarriers used in cell culture being discharged into the ocean through wastewater. Research in 2023 shows that 10^5 microplastic particles can be released per liter of culture medium. Additive synthesis (such as recombinant protein fermentation) may generate high-concentration organic wastewater, thereby polluting water sources. If the waste liquid contains unused carbon sources and nitrogen sources, it is prone to cause eutrophication of water bodies. However, although the overall carbon emissions of artificial meat are lower than those of traditional livestock farming, the production of new additives relies on high-energy-consuming processes such as 3D printing, which may offset its environmental benefits (Figure 1).

3.3. Comparison of Novel Additives in Plant-based Meat and Cell-cultured Meat

The current safety assessment methods for novel additives mainly include toxicological experiments and in vitro experiments, which are mainly focused on human health and ecological environment. By summarizing the existing research and experiments, the comprehensive safety and application development trends of novel additives in plant-based meat and cell-cultured meat are compared, as shown in Table 1.

Table 1. Comparison between Plant-based Meat and Cell-cultured Meat

	Types of artificial meat	Plant-based meat	Cell-cultured meat
Safety status and efficiency cost	Type of additives	Biosynthesis additive	
		Yeast fermentation of soybean hemoglobin	Recombinant factors (such as IGF-1)
	Acute toxicity test	Acute Oral Toxicity Test in Rats (OECD 423)	In vitro cytotoxicity (Caco-2 model)
	result	LD_{50} > 2000 mg/kg, No acute toxicity	High concentration (IC_{50} > 100 μg/mL) inhibits intestinal cell proliferation [17].
	Raw material cost	Glucose: 0.5 - 1.5/kg	Mammalian cell culture medium: 500 - 1000 g per liter
	R&d investment	$1 - $5 million per product	$10 - $50 million per product
	Type of additives	Functional additives	
		Bamboo leaf antioxidant (AOB)	Cellulose nanocrystals, plant-based scaffolds
	Subchronic toxicity test	90-day rat feeding study (OECD 408)	28-day mouse intake test
	Result	No pathological changes in the liver or kidneys [18].	Increased intestinal inflammation markers (IL-6) [19].
	Raw material cost	$0.2-0.8/kg	$50-200/kg
	Type of additives	Synthesize heme	Recombinant FGF-2
	Genetic toxicity test	Ame's test (OECD 471) + in vitro micronucleus test	Extracellular chromosome aberration test
	Result	Negative, no mutagenicity	High-dose-induced DNA damage [20].
	Type of additives	Methylcellulose, microencapsulated vegetable fats	Antibiotic residues in culture medium
	Residual risk	Detection of residual amount after processing by HPLC	LC-MS/MS analysis
	Result	Residual content < 0.1%, no biological accumulation [21]	Some batches were found to contain gentamicin.
Regulatory status		Most are GRAS-certified.	Only some countries have approved it (such as Singapore)
		The approval cycle is short, ranging from $0.5 to $2 million.	The approval cost is high - $5-10 million
Consumer acceptance		Over 70% of the global population are aware of plant-based products.	12% are familiar with cultured meat from cells
Market price		43% have major concerns about taste differences.	16% are concerned about the technical safety

Plant-based meat has become the dominant application trend, mainly manifested in comprehensive regulatory systems, high consumer recognition, and higher safety indices. Currently, cell-cultured meat is in the technical breakthrough stage and is facing new regulatory challenges, such as how to define "cell culture medium residues". The risks of sensitization and oxidative stress in plant-based meat can mostly be reduced through process optimization, while the bioactive molecules in cell-cultured meat may have unforeseeable interactions. Predicting for the next five to ten years, due to its lower cost and relatively mature experiments, new additives will account for more than 85% of the market share of alternative meat in plant-based artificial meat applications. According to the report on the trend analysis of the artificial meat industry in China in 2025 and the investment strategic planning, it is expected that by 2030, new additives can reduce the cost of cell-cultured meat by 70%, and the global market size will exceed 5 billion US dollars. In the future, the application of new additives in cell-cultured meat can mainly focus on special scenarios, such as space food, medical health products, and high-end customized meat.

4. Conclusion

The application of new additives has enhanced the taste and nutritional level of artificial meat products, but safety issues cannot be ignored. Animal experiments have preliminarily verified the safety of some additives, but there are differences between humans and animals, and current research is mostly limited to short-term or in vitro experiments. Essentially, developing new additives is the continuous exploration of human material performance boundaries and technical ethics. The innovation in the field of additives may give rise to new industrial forms, such as bio-manufacturing and molecular agriculture. However, its ultra-processed method and the composition of new additives determine that it is different from natural food. To ensure its safety for use, future research should focus on the development of high-tech, integrate knowledge from multiple fields such as toxicology, computational biology, food science and food safety, and prioritize the promotion of transparent labeling systems and life cycle environmental assessment, establishing a unified framework for human and ecological safety assessment and establishing a complete and improved database covering special populations.

Future research can fully utilize technological means such as artificial intelligence and big data to precisely control the application of additives. With the development of biotechnology, food engineering and materials science, the production technologies and pollution control technologies for new additives in artificial meat will become more mature and the costs will further decrease. With consumers' pursuit of food diversity, the types of new additives will become more diverse and their application in the artificial meat market will further expand. It is expected that in the future, it will present an internationalized development pattern, allowing high-quality artificial meat to enter thousands of households and become delicious delicacies on the dining tables of the general public.

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