| 1 |
|---|
| 2 |
| 3 |
| Λ |

TITLE PAGE - Food Science of Animal Resources -Upload this completed form to website with submission

| ARTICLE INFORMATION | Fill in information in each box below |
|--|---|
| Article Type | Research article |
| Article Title | Industrial research and development on the production process and quality of |
| | cultured meat hold significant value: a review |
| Running Title (within 10 words) | Improvement direction for cultured meat |
| Author | Kyu-Min Kang ¹ , Dong Bae Lee ^{3*} , and Hack-Youn Kim ^{1,2*} |
| Affiliation | ¹ Department of Animal Resources Science, Kongju National University, |
| | Chungnam 32439, Korea |
| | ² Resource Science Research Institute, Chungnam 32439, Korea |
| | ³ School of Languages and Cultures, the University of Queensland, Brisbane, |
| | QLD 4072, Australia |
| Special remarks – if authors have additional information to inform the editorial office | Not applicable. |
| ORCID (All authors must have ORCID) | Kyu-Min Kang (https://orcid.org/0000-0002-4904-1976) |
| https://orcid.org | Dong Bae Lee (https://orcid.org/0000-0003-2217-9227) Hack-Youn Kim (https://orcid.org/0000-0001-5303-4595) |
| Conflicts of interest List any present or potential conflict s of interest for all authors. (This field may be published.) | The authors declare no potential conflict of interest. |
| Acknowledgements | Not applicable. |
| State funding sources (grants, funding | |
| sources, equipment, and supplies). Include | |
| name and number of grant if available. (This field may be published.) | |
| Author contributions | Conceptualization: Kim HY, Lee DB. |
| (This field may be published.) | Investigation: Kang KM, Kim HY. |
| | Writing - original draft: Kang KM. |
| | Writing - review & editing: Kang KM, Lee DB, Kim HY. |
| Ethics approval (IRB/IACUC) | This article does not require IRB/IACUC approval because there are no humar |
| (This field may be published.) | and animal participants. |

7 CORRESPONDING AUTHOR CONTACT INFORMATION

| For the <u>corresponding</u> author (responsible for correspondence, proofreading, and reprints) | Fill in information in each box below | | |
|--|--|---|--|
| First name, middle initial, last name | Hack-Youn Kim, | Dong Bae Lee | |
| Email address – this is where your proofs will be sent | kimhy@kongju.ac.kr, | isaaclee@uq.edu.au | |
| Secondary Email address | | | |
| Postal address | Department of Animal Resources Science, Kongju National University, Chungnam 32439, Korea | School of Languages and Cultures, the University of Queensland, Brisbane, QLD 4072, Australia | |
| Cell phone number | | | |
| Office phone number | +82-41-330-1241, | +07-3365-6431 | |
| Fax number | +82-41-330-1249 | , +07-3365-6799 | |

10 Abstract

11 Cultured meat has been gaining popularity as a solution to the increasing problem of food insecurity. 12 Although research on cultured meat started later compared to other alternative meats, the industry is 13 growing rapidly every year, with developed products evaluated as being most similar to conventional meat. 14 Studies on cultured meat production techniques, such as culturing new animal cells and developing medium 15 sera and scaffolds, are being conducted intensively and diversely. However, active in-depth research on the 16 quality characteristics of cultured meat, including studies on the sensory and storage properties that directly 17 influence consumer preferences, is still lacking. Additionally, studies on the combination or ratio of fat cells 18 to muscle cells and on the improvement of microbiota, protein degradation, and fatty acid degradation 19 remain to be conducted. By actively investigating these research topics, we aim to verify the quality and 20 safety of cultured meats, ultimately improving the consumer preference for cultured meat products.

- 21
- 22 Keywords: Cultured meat, Manufacturing, Nutritional properties, Sensory properties, Storage properties
- 23
- 24

Introduction

25 With the recent increase in the global population, per capita gross domestic product (GDP) and meat 26 consumption are steadily increasing (Hong et al., 2021). The continual increase in meat consumption is 27 expected to increase the demand for staple meats, such as beef, pork, and chicken, by an average of 70% 28 by 2050 (Siddiqui et al., 2022A). Increased meat production is essential to meet such demand. However, 29 traditional and conventional livestock farming methods are becoming increasingly inadequate in meeting 30 this demand, owing to the requirements of large quantities of finite resources, such as land, water, and 31 grains (Guan et al., 2021). As a result, this situation is expected to lead to ongoing issues of food insecurity 32 and environmental problems (Goodwin and Shoulders, 2013). Therefore, some people have started to adopt 33 various forms of veganism as a dietary choice. This includes consumers classified as core vegans, trend-34 setting vegans, trend-following vegans, imperfect vegans, green vegans, and potential vegans (Treich,

35 2021). Moreover, plant-based proteins, insect proteins, and cultured meat are some of the products that
 36 have been researched and developed as alternatives to animal protein (Onwezen et al., 2021).

37 Cultured meat, also known as lab-grown meat, is the most recently developed alternative protein source. 38 It is produced by in vitro culturing of cells taken from the animal's body (Siddiqui et al., 2022B). Because 39 cultured meat is produced through cell cultivation in bioreactors, it has fewer ethical, religious, and 40 environmental constraints than meats produced by traditional livestock farming (Bryant, 2020). Therefore, 41 the commercialization of cultured meat in the protein market is anticipated to have a promising outlook and 42 offers advantages for introducing meats that are difficult to produce through traditional farming methods, 43 or are not commonly available, such as wild game (Lee et al., 2023). This development broadens the 44 diversity of food options for consumers. Furthermore, meat cultivation provides the potential to enhance 45 nutritional content and incorporate additives with various biofunctionalities, such as antioxidants and 46 anticancer and anti-inflammatory molecules, surpassing the benefits of consuming conventional meat 47 (Nobre, 2022).

However, globally integrated industrial regulations remain incomplete, and scientific research on this matter is also lacking. This suggests that cultured meat may be advantageous in helping to manage consumer health. Despite the fact that the cultured meat industry is advancing through various research and product development efforts, further validation of the products is required, particularly in terms of tissue texture and food safety (Ramani et al., 2021).

- 53
- 54
- 55

Manufacturing of cultured meat

56 **Donor selection**

57 Donor selection is the most fundamental aspect of the production process, involving considerations such 58 as the breed, sex, and age of the animal and the specific body part from which the cells are sourced (Stephens 59 et al., 2018). As shown in Table 1, cultured meat is being produced from cells sourced from various types 60 of animals. Currently, a significant number of commercialized products derived from this process have been developed and are available to consumers (Lee et al., 2022A). For these products, the cells are primarily sourced (in descending order of usage) from cattle (25%), poultry (22%), seafood (19%), pigs (19%), and other animals (15%) (Choudhury et al., 2020). Cattle and poultry are predominantly used for research purposes and most of those researches are targeted at religious consumers (Bryant, 2020).

65 Also, many consumers and scientists commonly know that cultured meat has high advantages for 66 religious reasons and the standard of cell selection is influenced by its reasons. However, for example in 67 the Islamic community, the main point of choosing meat is "Does the meat (cultured meat) produced follow 68 the halal status?" and this point shows that cultured meat isn't always suitable for religious people (Chriki 69 and Hocquette, 2020). Furthermore, Siddiqui et al. (2022B) reported that socially conservative consumers 70 expressed negative reactions towards cultured meat, and some religious communities, such as Hindus, 71 expressed vegetarianism is regarded as superior to meat eating. These discussions bring the new research 72 development of cell selection and collection techniques from animal bodies and many new studies have to 73 be started.

74 Once the livestock breed is selected, the next step involves selecting factors such as sex, age, and specific 75 parts of the animal. This decision is dictated by the quality of the satellite cells in the collected muscle tissue 76 (Skrivergaard et al., 2023), which is determined by assessing factors such as their yield and differentiation 77 capacity (Arshad et al., 2017). This assessment is conducted to select the most suitable tissue for meat 78 cultivation. Determining the quality of satellite cells is crucial because the cells play a pivotal role in the 79 regeneration of the muscle tissue that has been damaged through injury (Hong et al., 2021), making them 80 the most critical factor in the cell selection process. Kim et al. (2023A) reported that many factors (such as 81 gender, age, and environment) affect cultured meat production and there are existing unfigured mechanisms 82 that need research. Coles et al. (2015) reported that the breed of origin, live weight at slaughter, and carcass 83 weight affect the collected cell proliferation and this seems that differential gene expression is the main 84 reason for these phenomena.

For these reasons, the final product of cultured meat is affected by the cell donor animal's genetic characteristics, some researchers are proposing to establish optimized cell models in genetic engineering tools concerning genetically modified organisms (GMOs) (Martins et al., 2023). Also, some researchers found out that cultured meat is more suitable for their Swiss sample compared to GMOs food and this could be a key point for getting balance in the genetic engineering side of cultured meat (Bryant and Barnett, 2020). This describes that many new studies can be excavated in the donor selection part and could be additional scientific data for the traditional meat industry.

92

93 Cell isolation

Cell separation is the process by which the satellite cells are efficiently isolated from the muscle tissue (which comprises various cell types, including muscle fibers and stem cells) (Li et al., 2022A). This process ensures that only satellite cells are obtained from the tissue. Typically, after the initial separation through physical and chemical dissociation, secondary separation is performed using methods such as filtration and centrifugation, density gradient centrifugation, and cell separation based on the antigen–antibody reactions of surface markers (Swatler et al., 2020). Two commonly used cell separation methods are fluorescenceactivated cell sorting (FACS) and magnetic-activated cell sorting (MACS) (Table 2).

101 FACS utilizes antigen-antibody reactions to recognize surface markers on cells as antigens, which have 102 been pre-labeled with fluorescent substances to facilitate the cell sorting process. A flow cytometer is used 103 to separate the cells, allowing for the precise analysis of their size and internal structure (Kim et al., 2022A). 104 Furthermore, the integration of FACS with sequencing, known as FACSeq, proves to be highly effective. 105 This approach enables the detailed exploration of individual cell physiology, facilitating the identification 106 based on factors such as relative nucleic acid contents and cell membrane integrity (Dridi et al., 2023). 107 Recently, owing to the meticulous nature of the FACS method, certain researchers have devised a FACS 108 strategy specifically for purifying adipose progenitor cell (APC). Subsequently, they demonstrated that the 109 purified APC exhibited a notable capacity for proliferation and adipogenic differentiation (Song et al., 110 2022).

Similarly, MACS relies on antigen–antibody reactions, but antibodies with magnetic properties are used instead to react with antigens on the cell surface. Cells with attached antibodies are then separated using a magnet. This method facilitates rapid cell separation and high cell viability (Choi et al., 2020). Hence, MACS is considered less disruptive in the separation process compared to FACS, making it a more suitable choice for large-scale expansion (Kim et al., 2023B). While FACS incurs significant costs for both entry and maintenance and exhibits slow speed, hindering high-throughput sample handling, Bead-based MACS is a solution to these issues. Nonetheless, magnetic-based approaches grapple with challenges such as low specificity (stemming from the use of a single antibody type) and difficulties in scaling up samples due to the intricate relationship between magnetic field strength and distance (McNaughton et al., 2022).

Taking advantage of the strengths of both FACS and MACS, a hybrid approach that combines these two techniques for cell separation is being widely used in research pertaining to cultured meat production (Guan et al., 2022). In combining two techniques, the strengths of FACS, known for its multiple labeling and sorting capabilities, and MACS, appreciated for high throughput and quick sorting times. Kang et al. (2021) reported they developed an Immunomagnetic Microfluidic Integrated System (IM-MIS) that achieves high yield, high throughput, and minimal loss based on the differentiated cell phenotype.

With the ongoing advancements in these technologies, there is an anticipation that cell separation technology will stabilize, facilitating swift industrial progress in the field of cell sorting.

128

129 Cell culturing

130 Cell culturing primarily involves the use of proliferation methods to increase the number of selected cells 131 (Figure 1). Various substances, such as basal culture medium, serum, growth factors, and antibiotics, are 132 used to provide the necessary conditions for cell regeneration and maturation during this process (Siddiqui 133 et al., 2022B). Basic culture media, such as Dulbecco's modified Eagle's medium (DMEM), contain 134 essential nutrients to support and maintain the growth and health of the cells while exponentially increasing 135 their numbers. DMEM offers several advantages, such as commercial availability and a bio-mimicking 136 environment enriched with ingredients like amino acids and vitamins. Consequently, DMEM addresses 137 challenges associated with time-consuming preparation, as well as various issues related to precipitation 138 and storage (Bayrak et al., 2020).

Any deficiencies in the basic medium are supplemented with additives, such as a specific serum, growth
factors, and antibiotics (Zhang et al., 2020). Specifically, animal-derived sera, such as fetal bovine serum

141 (FBS), are crucial for cell cultures because they are highly effective in promoting cell proliferation (Post et 142 al., 2020). FBS, naturally tailored for the prenatal development of unborn calves, boasts an extensive array 143 of nutrients, growth factors, and adhesion factors with minimal antibody content (van der Valk, 2022). Its 144 historical preference stems from its relatively low cost and widespread availability, making FBS the primary 145 choice for supplementing nearly all eukaryotic cell culture media. However, demand for alternatives to sera 146 is growing, owing to the ethical concerns and high costs associated with their use. In recent years, various blood-free additives, such as B-27TM and Xerum FreeTM, have been developed to replace FBS (Guan et al., 147 148 2021). These products aim to minimize animal sacrifice and reduce the cost of cultured meat production. 149 Furthermore, to alleviate concerns regarding the consumption of antibiotics and anti-inflammatory agents 150 in the final cultured meat products, some producers have opted for methods that do not use these unwanted 151 bioactive molecules. However, this approach requires delicate culture control, as it can lead to a sharp 152 decrease in cell viability (Piochi et al., 2022).

Microcarriers, an optional material for cell culturing, are formed into beads and have been established as an expanded growth surface to support the differentiation and proliferation of various types of cultured cells (Norris et al., 2022). And there are edible, non-edible, and degradable microcarriers exist, among those kinds, edible microcarrier is most preferred and it is classified into polysaccharides, lipids, polypeptides, and composites/synthetics (Bodiou and Post, 2020). The importance of edible microcarriers is to reduce the final cost of cultured meat products by increasing cell harvest yield (Zernov et al., 2022).

159 The most critical environmental factor in cell culturing is temperature, as it is essential for cell 160 culturing. Mass cell culturing is predominantly carried out in bioreactors, where optimal cell 161 culture is conducted at a temperature of 37°C, mimicking the human body, and supplied with 162 oxygen (Garrison et al., 2022). Guan et al. (2022) reported that mildly elevated temperatures (39°C) 163 and mechanical stimulation are among the environmental cues that have been proven to boost both 164 myogenic differentiation and hypertrophy. Some environmental cues like mild high temperature (39°C) and mechanical movement have also been demonstrated to enhance myogenic 165 differentiation and hypertrophy (Guan et al., 2022). Consequently, while inducing heat stress 166

167 through elevated culture temperatures may not independently suffice for cell growth and 168 differentiation, it can effectively promote growth factor-mediated cell proliferation and 169 differentiation (Oh et al., 2023).

Taking these aspects into consideration, both in cell culture and collection, it becomes imperative to align
with the ethical consumption tendencies of consumers. Simultaneously, there is a continuous need to
explore avenues that provide industrial economic advantages.

173

174 Cell structuring

In cell structuring, the main point is to stabilize the differentiation of muscle cells. It is also called subsequent hypertrophy and this is the mix of biochemical and mechanical stimuli (Post, 2012). A scaffold structure is necessary for organizing the cultured cells into tissues. To reproduce all important features of conventional meat, the set of requirements for biomaterials used to produce cultured meat is highly specific (Wollschlaeger et al., 2022). The material should be edible, sustainable, widely available, animal-free, nontoxic, cheap, processable, and ideally have none or only a mild taste.

181 Animal-derived scaffolds, which are primarily composed of collagen, have the advantage of providing 182 minimal heterogeneity during cell cultivation. Furthermore, they contribute to the texture and flavor of the 183 final product, aiding in replicating the characteristics of conventional meat (Seah et al., 2022). Collagen 184 gels or collagen-Matrigel complexes are commonly used because they enhance protein production (Figure 185 4) (Post, 2012). Collagen stands out as a well-established material for cell adherent coatings in tissue 186 engineering. Considering that HC peptides share the identical amino acid sequence with collagen and retain 187 cell-binding capability even after collagen denaturation into gelatin, it is reasonable to anticipate robust cell 188 adhesion on hydrolyzed collagen surfaces (Koranne et al., 2022).

Plant-based scaffolds, which are existing plant structures onto which the cultured cells can be attached, offer the simplest means to achieving cellular myogenesis. Additionally, they allow for the consumption of nutrients naturally present in plants, providing an added advantage (Levi et al., 2022). Decellularized spinach is a representative plant-based scaffold that shows high cell adhesion and survival rate and forms suitable cost on the industrial side (Jones et al., 2021). To reproduce the structure of muscle tissues in decellularized spinach scaffolds, the critical factors include the precise composition of the tissue, the arrangement of cells within the scaffold, and the influence of surface topography and cell origin, which may vary based on plant species and leaf position (Rao et al., 2023). However, plant-based scaffolds, which may include polysaccharides such as cellulose, alginate, and hyaluronic acid, carry the risk of inducing allergies (Djisalov et al., 2021), rendering them less suitable for consumption by vulnerable consumers.

199 Recently, interest in the use of 3D printing technology has been growing, and research studies on the use 200 of 3D printers to produce scaffolds and to directly create cultured meat in the shape of conventional meat 201 are underway (Ramani et al., 2021). In 3D bioprinter, the nozzle size, extrusion pressure, and source of 202 filler highly affect the final products of cultured meat (Djisalov et al., 2021). The main strength of 3D 203 printing technology is the creation of free forms, allowing researchers to realize the desired shape with a 204 high realization rate and freely adjust the type and proportion of the structure (Li et al., 2021). Also 205 enhancing tissue distribution of macromolecules and cells, this technique contributes to producing final 206 products with improved organoleptic properties, offering precise deposition of cells, micronutrients, 207 technological aids, and biomaterials in predefined locations and shapes, presenting advantages over 208 alternative biofabrication methods (Barbosa et al., 2023).

While these aspects greatly aid in the differentiation of cells cultured on the scaffold into muscle, it seems essential to establish cell classification and safety verification methods that align with the scaffold's characteristics.

- 212
- 213
- 214

Quality properties of cultured meat

215 Nutritional properties

216 Various technological development studies have been conducted aiming to achieve comparable 217 nutritional components, such as protein, essential amino acids, vitamins, and mineral content, in cultured 218 meat compared to conventional meat, from a nutritional perspective (Fraeye et al., 2020). The nutritional

219 quality of cultured meat is influenced by the basic culture medium, serum, growth factors, and other 220 nutrients used in the cell culture. Various studies are underway to investigate the nutritional composition 221 and content of the products (Chriki and Hocquette, 2020). As of now, the protein content (the main reason 222 why people eat meats) of cultured meat has not been quantified; however, morphological observations 223 suggest similarities to traditional meat in terms of cytoskeletal proteins, with current research focusing on 224 optimizing the nutrient content of the growth medium to promote the development of cells with higher 225 protein content (Broucke et al., 2023). So huge differences appear in other nutrient contents except protein 226 contents between traditional meat and cultured meat.

227 The type and content of fat in cultured cells can be adjusted according to the manufacturer's preference 228 or purpose, and, like muscle cells, they must undergo a separate differentiation process during cultivation 229 (Fish et al., 2020). Fraeye et al. (2020) reported that the nature of the production process rendered regulation 230 of the fat composition of cultured meats possible, thus allowing for the development of healthier products 231 through adjustments of the essential fatty acid, polyunsaturated fatty acid, and trans-unsaturated fatty acid 232 ratios and calorie content. Accumulating as storage compounds in animal muscles, conventional meat is a 233 nutritionally dense food rich in high-quality proteins, as well as a diverse array of vitamins and minerals 234 (Singh et al., 2022). Meat blood is abundant in various nutrients, particularly minerals like calcium, iron, 235 magnesium, potassium, and sodium (Lee et al., 2022B). Therefore, consuming meat not only provides 236 essential nutrients directly but also includes minerals that are present in the blood.

237 However, in cultured meat, nutrient contents such as vitamins, minerals, etc. are affected by serum. The 238 composition and quantity of serum used can vary depending on the donor's biological information, diet, 239 and lifestyle (Lee et al., 2022C). Therefore, even the same type of serum can have differences in 240 components and amount. Kadim et al. (2015) reported that in cultured meat, the essential amino acids, 241 minerals, vitamins, and bioactive compounds provided by the basic culture medium, serum, and other 242 nutrients used during cell culture were similar to or even exceeded those in conventional meat, 243 demonstrating the nutritional advantages of meat cultivation. Currently, Ultroser G serves as a 244 commercially available serum-free growth medium, acting as a substitute for fetal bovine serum. It encompasses all the essential nutrients required for eukaryotic cell growth, including growth factors,binding proteins, adhesion factors, vitamins, hormones, and mineral trace elements (Jairath et al., 2021).

Therefore, cultured meat maintains its nutritional quality and can even contain enhanced contents of nutrients such as essential amino acids and fatty acids that may be lacking in conventional meat. The meat culturing process, thus, allows for the production of products with high nutritional value.

250

251 Textural properties

252 The latest research on textural properties has exposed suboptimal structuring and texture attributes in 253 manufactured cultured meat (Starowicz et al., 2022). Notably, non-instrumental studies profiling texture 254 has centered on sensory characteristics, including hardness, springiness, and chewiness (Yuliarti et al., 255 2021). Li et al. (2022B) reported that meat cultured on edible 3D chitosan-sodium alginate-collagen/gelatin 256 scaffolds had similar textural characteristics (e.g., chewiness, springiness, and resilience) as those of 257 conventional meat of the same weight, a finding they attributed to the comparable fibrous characteristics of 258 both products. Furthermore, in a study on cultured meat production using pig muscle stem cells, Zhu et al. 259 (2022) found that the addition of L-ascorbic acid 2-phosphate (Asc-2P) during the cell culture phase led to 260 increased expression of the myosin heavy chain protein and differentiation genes, which resulted in 261 enhancement of the tissue texture. Moreover, in their research on cultured meat using smooth muscle cells, 262 Zheng et al. (2021) observed that the texture of the final product was significantly influenced by the collagen 263 content. They found that the co-culturing of smooth muscle cells with hydrogel and formation of a network 264 structure enhanced the texture of cultured meat. This indicates that, aside from the characteristics of the 265 cultured cells themselves, the type of scaffold and additives used can also affect the texture of the final 266 product. Toivama et al. (2020) found that among various scaffold structures, those mimicking the striped 267 texture resembling muscle architecture promote myotube formation.

Also, some scaffolds can undergo breakdown and reconstruction by cells, in general, maintaining the structure and mechanical properties of the scaffold has a significant impact on the texture of cultured meat (Langelaan et al., 2010). In light of this, there is a trend in developing scaffolds using edible materials such as alginate, gelatin, collagen, and starch, taking advantage of the characteristics of the scaffold. Among

272 various scaffolds, animal-derived ones are suggested to more closely mimic the traditional texture of meat 273 compared to plant-based scaffolds (Levi et al., 2022). Paredes et al. (2022) compared the textural properties 274 of commercially available conventional sausages and sausages made from cultured meat and found that the 275 hardness, cohesiveness, springiness, chewiness, and gumminess of the two products were similar. This 276 finding suggests that cultured meat products are similar to conventional meat products in terms of textural 277 quality, highlighting the potential for future expansion into the development of cultured meat-based 278 products. However, in the case of cultured meat with a meat-like structure rather than a processed meat 279 form, currently available products for commercial sale have generally received lower consumer evaluations 280 compared to traditional meat (Kim et al., 2022B).

It is particularly suggested that ongoing efforts are needed for further improvement in texture, especiallyin terms of consistency.

283

284 Sensory properties

285 Intrinsic qualities such as taste, texture, smell, and nutritional value constitute the importance of meat. 286 These essential attributes play a critical role in influencing consumers' choices when it comes to purchasing 287 and consuming meat (Rombach et al., 2022). Furthermore, sensory properties are more treated as main 288 factors than price, health function, and convenience, and if the sensory properties are not well possessed, 289 consumer rejection rapidly increases (Pakseresht et al., 2022). The lipid oxidation products of conventional 290 meat interact with the products of the Maillard reaction, creating a complex flavor profile that contributes 291 to the meat color and taste (Chen et al., 2022). Therefore, for the flavor of conventional meat to be replicated 292 in cultured meat, an understanding of how well the product can mimic the taste of fats is needed (Ng and 293 Kurisawa, 2021).

Further research on the mechanisms of flavor compounds is necessary. Broucke et al. (2023) reported various studies that are using different methods to enhance the flavor of cultured meat, including coculturing adipocyte precursors with muscle cells and adding carotenoids during the cell culture phase, with a focus on flavor precursors. Additionally, Louis et al. (2023) investigated the regulation of the fatty acid composition in adipose-derived stem cells from Wagyu cattle and found that the initial lipid composition 299 can be controlled by adjusting the fatty acids during the cell differentiation process when producing fat 300 cells. This resulted in a fat composition similar to that of conventional meat. These studies indicate that a 301 foundation for replicating the flavor of fats in cultured meat has been established and underscore the need 302 for continued in-depth research specifically focusing on fat cells. Joo et al. (2022) conducted a comparative 303 study of cultured and conventional meats using electronic nose analysis. The researchers observed that 304 traditionally produced meat was superior in terms of flavors such as umami. Also, Rolland et al. (2020) 305 reported that a contrast in taste was evident between the conventional and 'cultured' hamburgers during the 306 sensory evaluation of six attributes, with the 'cultured' hamburger receiving a slightly favorable assessment. 307 This superiority was attributed to differences in the maturity of muscle fibers, implying that the flavor of 308 the final cultured meat can be influenced, even during the initial cell selection phase of primed cultivation. 309 All the above findings underscore the need for further research on the combinations and ratios of different 310 types of muscle and fat cells. Verbeke et al. (2015) reported that significant challenges lie in advancing 311 both the product and its production process to closely emulate traditional meat, especially concerning 312 sensory characteristics and pricing.

Additionally, challenges involve scaling up the process for enhanced resource efficiency and cost effectiveness, along with addressing regulatory and intellectual property issues.

315

316 Storage properties

Cultured meat is produced in a sterilized environment free of contaminants, making it generally safer than conventionally produced meat, in terms of microbial contamination. However, proper handling, processing, packaging, and storage practices after production need to be maintained (Siddiqui et al., 2022A). Upon introducing cultured meat to the market in the EU, regulations from the Genetically Modified Food and Feed Law have been applied, encompassing areas such as labeling, official control of animal-derived products, and microbiological criteria (Ketelings et al., 2021). Similar to other food production processes, ensuring safety throughout the entire cultured meat production process in the EU requires the 324 implementation of food safety monitoring systems like Hazard Analysis and Critical Control Points325 (HACCP).

326 Maintaining the storage stability of cultured meat serves not only the purpose of protecting consumers' 327 health from microorganisms but also aims to prevent changes in the texture characteristics of the final 328 product, which could impact the tissue structure (Rubio et al., 2020). Ong et al. (2023) reported that the 329 microbial composition of the final product is influenced by the indigenous microbial population in the 330 production environment. Therefore, the post-production microbial composition of cultured meat is 331 anticipated to be similar to that of the indigenous microbial population in the production environment. 332 Additionally, in their study on cultured meat with added carotenoids, Stout et al. (2020) found no significant 333 difference in malondialdehyde values between days 0 and 1 before heating of the regular cultured meat 334 samples; however, after heating, approximately two-fold difference was observed in malondialdehyde 335 values between days 0 and 1. This indicates that the storage conditions, form, and method greatly influence 336 the cultured meat after its production.

337 In particular, an analysis of the factors that lead to significant changes in meat stability after heating is 338 needed, and the implementation of appropriate storage methods is required. Furthermore, Singh et al. (2022) 339 reported that utilizing the fermentation characteristics of organisms such as mushrooms, yeast, and fungi 340 enhances the taste profile of cultured meat and extends its shelf life. This suggests that the use of natural 341 antimicrobials will increase in the future. Considering that cultured meat is primarily generated in a 342 laboratory environment, it can be regarded as less prone to zoonotic diseases than conventional meat 343 products. However, there are knowledge gaps in the current understanding of food safety concerning 344 cultured meat, particularly because the majority of research endeavors are concentrated on optimizing 345 production methods (Hardi and Brightwell, 2021).

346 Therefore, future research studies should focus on utilizing various additives to enhance the shelf life of 347 cultured meat while simultaneously improving other characteristics, such as flavor, texture, and nutrition.

348

Summary and future research

351 With the diversification of consumer preferences and increasing demand for meat, cultured meat is 352 gaining prominence as a future food resource. Various studies have been conducted on cultured meat 353 production, especially in the development of serum alternatives and scaffolding materials. With regard to 354 serum research, the development of artificial or blood-free serum cultivation methods has the potential to 355 reduce the final cost of cultured meat production. Regarding scaffolding materials, the utilization of 3D 356 printing techniques holds promise for enhancing both the speed and quality of cultured meat production. 357 Although there have been extensive studies on the nutritional quality and histological aspects of cultured 358 meats, research on their sensory and storage characteristics remains relatively limited. Considering that 359 these characteristics directly affect consumer preferences, continuous research and development in these areas are warranted. With regard to sensory characteristics, research on the combination and ratio of muscle 360 361 and fat cells is required to achieve a flavor similar to that of traditional meat. Furthermore, studies on the 362 storage conditions, forms, and packaging methods are required to maintain the freshness and safety of 363 cultured meats and their products. Specifically, studies on hygiene-related aspects (for instance, microbial 364 composition), lipid oxidation, and protein degradation are crucial to demonstrate the practicality of cultured 365 meats. Such research endeavors are expected to contribute greatly to improving consumer preferences for 366 these products in the future. Furthermore, it appears that ongoing research with sample weights similar to 367 actual meat is imperative to enhance industrial relevance and value. In the future of cultured meat, research 368 at the product level, focusing on weights comparable to finished products, should persist to ensure 369 continuous elevation of industrial value and advancement. This task will likely become a focal point for 370 researchers in the field.

371

372 **Conflict of interest**

373 The authors declare no potential conflicts of interest.

374

375 Acknowledgments

- Not applicable.
- 377
- 378 Author contributions
- 379 Conceptualization: Kim HY, Lee DB.
- 380 Investigation: Kang KM, Kim HY.
- 381 Writing original draft: Kang KM.
- 382 Writing review & editing: Kang KM, Lee DB, Kim HY.
- 383
- 384 **Ethics Approval**
- 385 This article does not require IRB approval because there are no human and animal participants.

| 387 | References |
|-----|---|
| 388 | Arshad MS, Javed M, Sohaib M, Saeed F, Imran A, Amjad Z. 2017. Tissue engineering approaches to |
| 389 | develop cultured meat from cells: a mini review. Cogent Food Agric 3:1320814. |
| 390 | Bain PA, Hutchinson RG, Marks AB, Crane MSJ, Schuller KA. 2013. Establishment of a continuous cell |
| 391 | line from southern bluefin tuna (Thunnus maccoyii). Aquacult 376:59-63. |
| 392 | Barbosa W, Correia P, Vieira J, Leal I, Rodrigues L, Nery T, Barbosa J, Soares, M. 2023. Trends and |
| 393 | technological challenges of 3D bioprinting in cultured meat: technological prospection. Appl Sci |
| 394 | 13:12158. |
| 395 | Bayrak Ö, Ghahramanzadeh Asl H, Ak A. 2020. Comparison of SBF and DMEM in terms of |
| 396 | electrochemical properties of common metallic biomaterials. Mater Corros 71:209-221. |
| 397 | Bodiou V, Moutsatsou P, Post M J. 2020. Microcarriers for upscaling cultured meat production. Front Nutr |
| 398 | 7:10. |
| 399 | Broucke K, Van Pamel E, Van Coillie E, Herman L, Van Royen G. 2023. Cultured meat and challenges |
| 400 | ahead: a review on nutritional, technofunctional and sensorial properties, safety and legislation. Meat |
| 401 | sci 195:109006. |
| 402 | Bryant CJ. Culture, meat, and cultured meat. 2020. J Anim Sci 98:skaa172. |
| 403 | Bryant C, Barnett J. 2020. Consumer acceptance of cultured meat: an updated review (2018–2020). Appl |
| 404 | Scie 10:5201. |
| 405 | Carpenter CE, Rodriguez BT, Cockett NE. 2000. Growth and differentiation of cultured satellite cells from |
| 406 | callipyge and normal lambs. Canadian J Anim Sci 80:297-302. |
| 407 | Chen YP, Feng X, Blank I, Liu Y. 2022. Strategies to improve meat-like properties of meat analogs meeting |
| 408 | consumers' expectations. Biomaterials 287:121648. |
| 409 | Choi KH, Kim M, Yoon JW, Jeong J, Ryu M, Jo C, Lee CK. 2020. Purification of pig muscle stem cells |
| 410 | using magnetic-activated cell sorting (MACS) based on the expression of cluster of differentiation |
| 411 | 29 (CD29). Food Sci Anim Resour 40:852. |
| | |
| | |

- Choi KH, Yoon JW, Kim M, Jeong J, Ryu M, Park S, Jo C, Lee CK. 2020. Optimization of culture
 conditions for maintaining pig muscle stem cells in vitro. Food Sci Anim Resour 40:659.
- 414 Choudhury D, Tseng TW, Swartz E. 2020. The business of cultured meat. Trends Biotechnol 38:573-577.
- 415 Chriki S, Hocquette JF. 2020. The myth of cultured meat: a review. Front Nutr 7:7.
- Clark DL, Coy CS, Strasburg GM, Reed KM, Velleman SG. 2016. Temperature effect on proliferation and
 differentiation of satellite cells from turkeys with different growth rates. Poult Sci 95:934-947.
- 418 Coles CA, Wadeson J, Leyton CP, Siddell JP, Greenwood PL, White JD, McDonagh MB. 2015.
- 419 Proliferation rates of bovine primary muscle cells relate to liveweight and carcase weight in cattle.
 420 PLoS One 10:e0124468.
- 421 Djisalov M, Knežić T, Podunavac I, Živojević K, Radonic V, Knežević NŽ, Bobrinetskiy I, Gadjanski I.
- 422 2021. Cultivating multidisciplinarity: manufacturing and sensing challenges in cultured meat
 423 production. Biology 10:204.
- 424 Dridi L, Altamura F, Gonzalez E, Lui O, Kubinski R, Pidgeon R, Montagut A, Chong J, Xia J, Maurice C
- F, Castagner B. 2023. Identifying glycan consumers in human gut microbiota samples using
 metabolic labeling coupled with fluorescence-activated cell sorting. Nat Commun 14:662.
- Fish KD, Rubio NR, Stout AJ, Yuen JS, Kaplan DL. 2020. Prospects and challenges for cell-cultured fat as
 a novel food ingredient. Trends Food Sci Technol 98:53-67.
- Fraeye I, Kratka M, Vandenburgh H, Thorrez L. 2020. Sensorial and nutritional aspects of cultured meat
 in comparison to traditional meat: much to be inferred. Front Nutr 7:35.
- 431 Fülber J, Agreste FR, Seidel SR, Sotelo ED, Barbosa ÂP, Michelacci YM, Baccarin RY. 2021.
 432 Chondrogenic potential of mesenchymal stem cells from horses using a magnetic 3D cell culture
 422 August Magnetic Letter Cells 12:645
- 433 system. World J Stem Cells 13:645.
- Furuhashi M, Morimoto Y, Shima A, Nakamura F, Ishikawa H, Takeuchi S. 2021. Formation of contractile
 3D bovine muscle tissue for construction of millimetre-thick cultured steak. Sci Food 5:6.
- 436 Garrison GL, Biermacher JT, Brorsen BW. 2022. How much will large-scale production of cell-cultured
- 437 meat cost?. J Agric Food Res 10:100358.

- Goodwin JN, Shoulders CW. 2013. The future of meat: a qualitative analysis of cultured meat media
 coverage. Meat Sci 95:445-450.
- Guan X, Lei Q, Yan Q, Li X, Zhou J, Du G, Chen J. 2021. Trends and ideas in technology, regulation and
 public acceptance of cultured meat. Future Foods 3:100032.
- Guan X, Zhou J, Du G, Chen J. 2022. Bioprocessing technology of muscle stem cells: implications for
 cultured meat. Trends Biotechnol 40:721-734.
- Guan X, Yan Q, Ma Z, Zhou J. 2023. Production of mature myotubes in vitro improves the texture and
 protein quality of cultured pork. Food Funct 14:3576-3587.
- 446 Hadi J, Brightwell G. 2021. Safety of alternative proteins: Technological, environmental and regulatory
- 447 aspects of cultured meat, plant-based meat, insect protein and single-cell protein. Foods 10:1226.
- Hong TK, Shin DM, Choi J, Do JT, Han SG. 2021. Current issues and technical advances in cultured meat
 production: a review. Food Sci Anim Resour 41:355.
- Jairath G, Mal G, Gopinath D, Singh B. 2021. A holistic approach to access the viability of cultured meat:
 A review. Trends Food Sci Technol 110:700-710.
- Jang M, Scheffold J, Bruheim P. 2022. Isolation and cultivation of primary muscle cells from Lobster
 (Homarus gammarus). In Vitro Cell Dev Biol Anim 58:446-451.
- Jones JD, Rebello AS, Gaudette GR. 2021. Decellularized spinach: An edible scaffold for laboratory-grown
 meat. Food Biosci 41:100986.
- 456 Joo ST, Choi JS, Hur SJ, Kim GD, Kim CJ, Lee EY, Bakhsh A, Hwang YH. 2022. A comparative study
- 457 on the taste characteristics of satellite cell cultured meat derived from chicken and cattle muscles.
 458 Food Sci Anim Resour 42:175.
- Kadim IT, Mahgoub O, Baqir S, Faye B, Purchas R. 2015. Cultured meat from muscle stem cells: a review
 of challenges and prospects. J Integr Agric 14:222-233.
- Ketelings L, Kremers S, de Boer A. 2021. The barriers and drivers of a safe market introduction of cultured
 meat: a qualitative study. Food Control 130:108299.
- 463 Kim SH, Kim CJ, Lee EY, Son YM, Hwang YH, Joo ST. 2022A. Optimal pre-plating method of chicken
- 464 satellite cells for cultured meat production. Food Sci Anim Resour 42:942-952.

- Kim B, Ko D, Choi SH, Park S. 2023B. Bovine muscle satellite cells in calves and cattle: a comparative
 study of cellular and genetic characteristics for cultivated meat production. Curr Res Food Sci
 7:100545.
- 468 Kim CJ, Kim SH, Lee EY, Son YM, Bakhsh A, Hwang YH, Joo ST. 2023C. Optimal temperature for
- 469 culturing chicken satellite cells to enhance production yield and umami intensity of cultured meat.
- 470 Food Chem Adv 2:100307.

- 471 Kim S, Beier A, Schreyer HB, Bakshi BR. 2022B. Environmental life cycle assessment of a novel cultivated
 472 meat burger patty in the united states. Sustainability 14:16133.
- 473 Kim Y, Oh S, Park G, Park S, Park Y, Choi H, Kim M, Choi J. 2023A. Characteristics of bovine muscle
- 474 satellite cell from different breeds for efficient production of cultured meat. J Anim Sci Technol.
- 476 Exploring properties of edible hydrolyzed collagen for 3D food printing of scaffold for
 477 biomanufacturing cultivated meat. Procedia CIRP 110:186-191.

Koranne V, Jonas OLC, Mitra H, Bapat S, Ardekani AM, Sealy MP, Rajukar K, Malshe AP. 2022.

- 478 Langelaan ML, Boonen KJ, Polak RB, Baaijens FP, Post MJ, van der Schaft DW. 2010. Meet the new meat:
 479 tissue engineered skeletal muscle. Trends Food Sci Technol 21:59-66.
- 480 Lee DY, Lee SY, Jung JW, Kim JH, Oh DH, Kim HW, Kang JH, Choi JS, Kim GD, Joo ST, Hur SJ. 2022A.
- 481 Review of technology and materials for the development of cultured meat. Crit Rev Food Sci Nutr.
 482 1-25.
- Lee DY, Lee SY, Yun SH, Jeong JW, Kim HW, Choi JS, Kim GD, Joo ST, Choi I, Hur SJ. 2022C. Review
 of the current research on fetal bovine serum and the development of cultured meat. Food Sci Anim
 Resour 42:775.
- Lee SY, Lee DY, Jeong JW, Kim JH, Yun SH, Joo ST, Choi I, Choi JS, Kim GD, Hur SJ. 2023. Studies on
 meat alternatives with a focus on structuring technologies. Food Bioprocess Technol 16:1389-1412.
- 488 Lee SY, Yun SH, Jeong JW, Kim JH, Kim HW, Choi JS, Kim GD, Joo ST, Choi I, Hur SJ. 2022B. Review
- 489 of the current research on fetal bovine serum and the development of cultured meat. Food Sci Anim490 Resour 42:775.
 - 21

- 491 Levi S, Yen FC, Baruch L, Machluf M. 2022. Scaffolding technologies for the engineering of cultured meat:
 492 towards a safe, sustainable, and scalable production. Trends Food Sci Technol 126:13-25.
- 493 Li L, Chen L, Chen X, Chen Y, Ding S, Fan X, Liu Y, Xu X, Zhou G, Zhu B, Ullah N, Feng X. 2022B.
- 494 Chitosan-sodium alginate-collagen/gelatin three-dimensional edible scaffolds for building a 495 structured model for cell cultured meat. Int J Biol Macromol 209:668-679.
- Li M, Wang D, Fang J, Lei Q, Yan Q, Zhou J, Chen J, Guan X. 2022A. An efficient and economical way
 to obtain porcine muscle stem cells for cultured meat production. Food Res Int 162:112206.
- Li Y, Liu W, Li S, Zhang M, Yang F, Wang S. 2021. Porcine skeletal muscle tissue fabrication for cultured
 meat production using three-dimensional bioprinting technology. J Future Foods 1:88-97.
- 500 Louis F, Furuhashi M, Yoshinuma H, Takeuchi S, Matsusaki M. 2023. Mimicking wagyu beef fat in
- 501 cultured meat: progress in edible bovine adipose tissue production with controllable fatty acid 502 composition. Mater Today Bio 21:100720.
- Luo W, Geng Y, Gao M, Cao M, Wang J, Yang J, Sun C, Yan X. 2022. Isolation and identification of bone
 marrow mesenchymal stem cells from forest musk deer. Animals 13:17.
- Martins B, Bister A, Dohmen RG, Gouveia MA, Hueber R, Melzener L, Messmer T, Papadopoulos J,
 Pimenta J, Raina D, Schaeken L, Shirley S, Bouchet BP, Flack JE. 2023. Advances and challenges
 in cell biology for cultured meat. Annu Rev Anim Biosci 12.
- McNaughton BH, Anker JN, Kinnunen PK. 2022. Buoyant-Antigen-Magnetic (BAM) immunoseparation,
 isolation, and detection of specific pathogenic bacterial cells. BioRxiv 2022-11.
- 510 Messmer T, Dohmen RG, Schaeken L, Melzener L, Hueber R, Godec M, Didoss C, Post MJ, Flack JE.
- 511 2023. Single-cell analysis of bovine muscle-derived cell types for cultured meat production. Front
 512 Nutr 10.
- Ng S, Kurisawa M. 2021. Integrating biomaterials and food biopolymers for cultured meat production. Acta
 Biomater 124:108-129.
- 515 Nobre FS. 2022. Cultured meat and the sustainable development goals. Trends Food Sci Technol. 124:140516 153.

- Norris SC, Kawecki NS, Davis AR, Chen KK, Rowat AC. 2022. Emulsion-templated microparticles with
 tunable stiffness and topology: applications as edible microcarriers for cultured meat. Biomater
 287:121669.
- 520 Oh S, Park S, Park Y, Kim YA, Park G, Cui X, Kim K, Joo S, Hur S, Kim G, Choi J. 2023. Culturing
- characteristics of Hanwoo myosatellite cells and C2C12 cells incubated at 37° C and 39° C for
 cultured meat. J Anim Sci Technol 65:664.
- 523 Okamoto Y, Haraguchi Y, Yoshida A, Takahashi H, Yamanaka K, Sawamura N, Asahi T, Shimizu T. 2022.
 524 Proliferation and differentiation of primary bovine myoblasts using Chlorella vulgaris extract for
- 525 sustainable production of cultured meat. Biotechnol Progr 38:e3239.
- 526 Ong KJ, Tejeda-Saldana Y, Duffy B, Holmes D, Kukk K, Shatkin JA. 2023. Cultured meat safety research
- 527 priorities: regulatory and governmental perspectives. Foods 12:2645.
- Onwezen MC, Bouwman EP, Reinders MJ, Dagevos H. 2021. A systematic review on consumer acceptance
 of alternative proteins: pulses, algae, insects, plant-based meat alternatives, and cultured meat.
 Appetite 159:105058.
- 531 Pakseresht A, Kaliji SA, Canavari M. 2022. Review of factors affecting consumer acceptance of cultured
 532 meat. Appetite 170:105829.
- 533 Paredes J, Cortizo-Lacalle D, Imaz AM, Aldazabal J, Vila M. 2022. Application of texture analysis methods
 534 for the characterization of cultured meat. Sci Rep 12:3898.
- 535 Park J, Lee J, Song KD, Kim SJ, Kim DC, Lee SC, Son YJ, Choi HW, Shim K. 2021. Growth factors
- improve the proliferation of Jeju black pig muscle cells by regulating myogenic differentiation 1 andgrowth-related genes. Anim Biosci 34:1392.
- 538 Pasitka L, Cohen M, Ehrlich A, Gildor B, Reuveni E, Ayyash M, Wissotsky G, Herscovici A, Kaminker R,
- 539 Niv A, Bitcover R, Dadia O, Rudik A, Voloschin A, Shimoni M, Cinnamon Y, Nahmias Y. 2023.
- 540 Spontaneous immortalization of chicken fibroblasts generates stable, high-yield cell lines for serum-
- 541 free production of cultured meat. Nat Food 4:35-50.
- 542 Perruchot MH, Ecolan P, Sorensen IL, Oksbjerg N, Lefaucheur L. 2012. In vitro characterization of
- 543 proliferation and differentiation of pig satellite cells. Differ 84:322-329.

- 544 Piochi M, Micheloni M, Torri L. 2022. Effect of informative claims on the attitude of Italian consumers
- towards cultured meat and relationship among variables used in an explicit approach. Food Res Int151:110881.
- 547 Post MJ, Levenberg S, Kaplan DL, Genovese N, Fu J, Bryant CJ, Negowetti N, Verzijden K, Moutsatsou
- 548 P. 2020. Scientific, sustainability and regulatory challenges of cultured meat. Nat Food. 1:403–415.
- 549 Post MJ. 2012. Cultured meat from stem cells: challenges and prospects. Meat Sci 92:297-301.
- 550 Promtan P, Panatuk J, Kongbuntad W, Amornlerdpison D, Nanta Y, Pripwai N, Thaworn W, Pattanawong
- W. 2023. Growth and development of black-boned chicken embryonic stem cells for culture meat
 using different serums as medium. Fac Anim Sci 46:354-360
- Ramani S, Ko D, Kim B, Cho C, Kim W, Jo C, Lee CK, Kang J, Hur S, Park S. 2021. Technical
 requirements for cultured meat production: a review. J Anim Sci Technol 63:681.
- Rao KM, Choi SM, Han SS. 2023. A review on directional muscle cell growth in scaffolding biomaterials
 with aligned porous structures for cultivated meat production. Food Res Int 112755.
- Rolland NC, Markus CR, Post MJ. 2020. The effect of information content on acceptance of cultured meat
 in a tasting context. PLoS One 15:e0231176.
- 559 Rombach M, Dean D, Vriesekoop F, de Koning W, Aguiar LK, Anderson M, Mongondry P, Oppong-
- 560 Gyamfi M, Urbano B, Luciano CAG, Hao W, Eastwick E, Jiang Z, Boereboom A. 2022. Is cultured
- 561 meat a promising consumer alternative? Exploring key factors determining consumer's willingness
- to try, buy and pay a premium for cultured meat. Appetite 179:106307.
- Rubio NR, Xiang N, Kaplan DL. 2020. Plant-based and cell-based approaches to meat production. Nat
 Commun 11:6276.
- 565 Saadeldin IM, Swelum AAA, Noreldin AE, Tukur HA, Abdelazim AM, Abomughaid MM, Alowaimer AN.
- 566 2019. Isolation and culture of skin-derived differentiated and stem-like cells obtained from the567 Arabian camel (Camelus dromedarius). Animals 9:378.
- 568 Seah JSH, Singh S, Tan LP, Choudhury D. 2022. Scaffolds for the manufacture of cultured meat. Crit Rev
- 569 Biotechnol 42:311-323.

- 570 Siddiqui SA, Bahmid NA, Karim I, Mehany T, Gvozdenko AA, Blinov AV, Nagdalian AA, Arsyad M,
- 571 Lorenzo JM. 2022A. Cultured meat: processing, packaging, shelf life, and consumer acceptance.
 572 LWT 172:114192.
- 573 Siddiqui SA, Khan S, Murid M, Asif Z, Oboturova NP, Nagdalian AA, Blinov AV, Ibrahim SA, Jafari SM.
- 574 2022B. Marketing strategies for cultured meat: a review. Appl Sci 12:8795.
- 575 Singh S, Yap WS, Ge XY, Min VLX, Choudhury D. 2022. Cultured meat production fuelled by
 576 fermentation. Trends Food Sci Technol 120:48-58.
- 577 Singh S, Yap WS, Ge XY, Min VLX, Choudhury D. 2022. Cultured meat production fuelled by
 578 fermentation. Trends Food Sci Technol 120:48-58.
- 579 Skrivergaard S, Rasmussen MK, Sahebekhtiari N, Young JF, Therkildsen M. 2023. Satellite cells sourced
- from bull calves and dairy cows differs in proliferative and myogenic capacity–Implications for
 cultivated meat. Food Res Int 173:113217.
- Song WJ, Liu PP, Meng ZQ, Zheng YY, Zhou GH, Li HX, Ding SJ. 2022. Identification of porcine adipose
 progenitor cells by fluorescence-activated cell sorting for the preparation of cultured fat by 3D
 bioprinting. Food Res Int 162:111952.
- Starowicz M, Poznar KK, Zieliński H. 2022. What are the main sensory attributes that determine the
 acceptance of meat alternatives?. Curr Opin Food Sci 100924.
- Stephens N, Di Silvio L, Dunsford I, Ellis M, Glencross A, Sexton A. 2018. Bringing cultured meat to
 market: technical, socio-political, and regulatory challenges in cellular agriculture. Trends Food Sci
 Technol 78:155-166.
- Stout AJ, Mirliani AB, Soule-Albridge EL, Cohen JM, Kaplan DL. 2020. Engineering carotenoid
 production in mammalian cells for nutritionally enhanced cell-cultured foods. Metab Eng 62:126137.
- Stout AJ, Mirliani AB, Rittenberg ML, Shub M, White EC, Yuen Jr, Kaplan DL. 2022. Simple and effective
 serum-free medium for sustained expansion of bovine satellite cells for cell cultured meat. Commun
 Biol 5:466.

- Sui M, Zheng Q, Wu H, Ding J, Liu Y, Li W, Chu M, Zhang Z, Ling Y. 2018. Isolation, culture and
 myogenic differentiation of muscle stem cells in goat fetal. Sci Agric Sin 51:1590-1597.
- Swatler J, Dudka W, Piwocka K. 2020. Isolation and characterization of extracellular vesicles from cell
 culture conditioned medium for immunological studies. Curr Protocol Immunol 129:e96.
- 600 Tomiyama AJ, Kawecki NS, Rosenfeld DL, Jay JA, Rajagopal D, Rowat AC. 2020. Bridging the gap
- between the science of cultured meat and public perceptions. Trends Food Sci Technol 104:144-152.
- Treich N. 2021. Cultured meat: promises and challenges. Environ Resour Econo 79:33-61.
- 603 van der Valk J. 2022. Fetal bovine serum—a cell culture dilemma. Sci 375:143-144.
- Vegusdal A, Sundvold H, Gjøen T, Ruyter B. 2003. An in vitro method for studying the proliferation and
 differentiation of Atlantic salmon preadipocytes. Lipids 38:289-296.
- 606 Verbeke W, Sans P, Van Loo EJ. 2015. Challenges and prospects for consumer acceptance of cultured meat.
 607 J Integr Agric 14:285-294.
- Wang S, Zhang Y, Xu Q, Yuan X, Dai W, Shen X, Wang Z, Chang G, Wang Z, Chen G. 2018. The
 differentiation of preadipocytes and gene expression related to adipogenesis in ducks (Anas
 platyrhynchos). PLoS One 13:e0196371.
- 611 Wollschlaeger JO, Maatz R, Albrecht FB, Klatt A, Heine S, Blaeser A, Kluger PJ. 2022. Scaffolds for
- 612 cultured meat on the basis of polysaccharide hydrogels enriched with plant-based proteins. Gels 8:94.
- 613 Yuliarti O, Kovis TJK, Yi NJ. 2021. Structuring the meat analogue by using plant-based derived composites.
 614 J Food Eng 288:110138.
- 615 Zernov A, Baruch L, Machluf M. 2022. Chitosan-collagen hydrogel microparticles as edible cell
 616 microcarriers for cultured meat. Food Hydrocolloids 129:107632.
- 617 Zhang G, Zhao X, Li X, Du G, Zhou J, Chen J. 2020. Challenges and possibilities for bio-manufacturing
 618 cultured meat. Trends Food Sci Technol 97:443-450.
- 619 Zhang S, Lou H, Lu H, Xu E, Liu D, Chen Q. 2023. Characterization of proliferation medium and Its effect
- on differentiation of muscle satellite cells from larimichthys crocea in cultured fish meat production.
- 621 Fishes 8:429.

- Zhao Y, Guo L, Guo H. 2023. Routine development of long-term primary cell culture and finite cell line
 from the hemolymph of greasyback shrimp (Metapenaeus ensis) and virus susceptibility. Aquacult
 563:739007.
- 625 Zheng YY, Zhu HZ, Wu ZY, Song WJ, Tang CB, Li CB, Ding SJ, Zhou GH. 2021. Evaluation of the effect
- of smooth muscle cells on the quality of cultured meat in a model for cultured meat. Food Res Int150:110786.
- 628 Zhu H, Wu Z, Ding X, Post MJ, Guo R, Wang J, Wu J, Tang W, Zhou G. 2022. Production of cultured
 629 meat from pig muscle stem cells. Biomater 287:121650.
- 630 Zhu G, Gao D, Li L, Yao Y, Wang Y, Zhi M, Zhang J, Chen X, Zhu Q, Gao J, Chen T, Zhang X, Wang T,
- 631 Cao S, Ma A, Feng X, Han J. 2023. Generation of three-dimensional meat-like tissue from stable pig
- epiblast stem cells. Nat Commun 14:8163.



Tables and Figures

634

635

| Bovine | Simmental Japanese black | Primary bovine satellite cells | Muscle tissue form | Stout et al. |
|-----------|---|-----------------------------------|--------------------|---------------------------|
| | Japanese black | | | (2022) |
| | I | Bovine myocytes | Steak form | Furuhashi et al (2021) |
| | Belgian Blue | Mixed cells | Muscle tissue form | Messmer et al (2023) |
| | Jeju black | Satellite cells | Muscle tissue form | Kim et al. (2023) |
| | Holstein Friesian | Peri-renal adipose cells | Fat tissue form | Okamoto et al (2022) |
| Swine | LYD (Landrace×Yorkshire ×Duroc) | Muscle stem cells | Muscle tissue form | Choi et al. (2020) |
| | Nongda Xiang | Muscle stem cells | Muscle tissue form | Zhu et al. (2023) |
| | Jeju black | Muscle stem cells | Muscle tissue form | Park et al. (2021) |
| | Pietrain X (Large White×Landrace) | Satellite cells | Muscle tissue form | Perruchot et al (2012) |
| | Large white | Satellite cells | Steak form | Guan et al. (2023) |
| Poultry | Hy-line brown (Chicken) | Satellite cells | Muscle tissue form | Kim et al. (2023C) |
| | Broiler Ross (Chicken) | Primary fibroblast cells | Steak form | Pasitka et al. (2023) |
| | Black-bone (Chicken) | Embryonic stem cells | Muscle tissue form | Promtan et al. (2023) |
| | Cherry Valley White-crested Jianchang (Duck) | Pre-adipocytes cells | Fat tissue form | Wang et al. (2018) |
| | Turkey | Satellite cells | Muscle tissue form | Clark et al. (2016) |
| Mammalian | Sheep | Satellite cells | Muscle tissue form | Carpenter et al |

636 Table 1. Types of cell donors for manufacturing cultured meat

| | | | | (2000) |
|---------|----------------------|-------------------------|--------------------------|---------------------------|
| | Goat | Muscle stem cells | Muscle tissue form | Sui et al. (2018) |
| | Horse | Mesenchymal stem cells | Chondrogenic tissue form | Fülber et al. (2021) |
| | Camel | Skin fibroblasts cells | Skin tissue form | Saadeldin et al. (2019) |
| | Deer | Mesenchymal stem cells | Muscle tissue form | Luo et al. (2022) |
| Fishery | Atlantic salmon | Adipose cells | Fat tissue form | Vegusdal et al. (2003) |
| | Large yellow croaker | Piscine satellite cells | Muscle tissue form | Zhang et al. (2023) |
| | Bluefin tuna | Cells | Tissue form | Bain et al. (2013) |
| | Greasyback shrimp | Cells | Tissue form | Zhao et al. (2023) |
| | Lobster | Primary muscle cells | Muscle tissue form | Jang et al. (2022) |

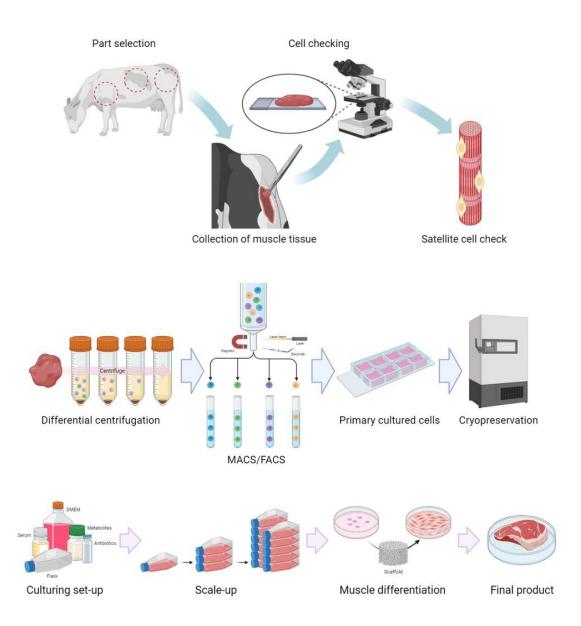
| 1 able 2. Differences of cell isolation methods | 639 | Table 2. Differences of cell isolation r | nethods |
|---|-----|--|---------|
|---|-----|--|---------|

| Characteristics | FACS | MACS | Hybrid |
|---|-----------------|--------------------------|-----------------|
| Surface antigens | Not essential | Essential | Not essential |
| Fluorescence cell labeling | Required | Not Required | Required |
| Cell purity | High | Medium | High |
| Concurrent categorization of diverse groups | Possible | Not possible | Possible |
| Categorizing by varied levels of expression | Possible | Not Possible | Possible |
| Cell separation | Trypsinize | Magnetic | Complex |
| Positive selection | Possible | Possible | Possible |
| Negative selection | Possible | Possible (low purity) | Possible |
| Multi marker selection | Possible | Very limited | Possible |
| Operation specificity | High | High | High |
| Equipment price | High | Low | High |
| Technical proficiency | Highly required | Low required | Highly required |

| Cell source | Breed | Cell kind | Product form | Reference |
|-------------|---|-----------------------------------|---------------------|---------------------------|
| Bovine | Simmental | Primary bovine satellite cells | Muscle tissue form | Stout et al. (2022) |
| | Japanese black | Bovine myocytes | Steak form | Furuhashi et al (2021) |
| | Belgian Blue | Mixed cells | Muscle tissue form | Messmer et al. (2023) |
| | Jeju black | Satellite cells | Muscle tissue form | Kim et al. (2023) |
| | Holstein Friesian | Peri-renal adipose cells | Fat tissue form | Okamoto et al. (2022) |
| Swine | LYD (Landrace×Yorkshire ×Duroc) | Muscle stem cells | Muscle tissue form | Choi et al. (2020) |
| | Nongda Xiang | Muscle stem cells | Muscle tissue form | Zhu et al. (2023) |
| | Jeju black | Muscle stem cells | Muscle tissue form | Park et al. (2021) |
| | Pietrain X (Large White×Landrace) | Satellite cells | Muscle tissue form | Perruchot et al (2012) |
| | Large white | Satellite cells | Steak form | Guan et al. (2023) |
| Poultry | Hy-line brown (Chicken) | Satellite cells | Muscle tissue form | Kim et al. (2023C) |
| | Broiler Ross (Chicken) | Primary fibroblast cells | Steak form | Pasitka et al. (2023) |
| | Black-bone (Chicken) | Embryonic stem cells | Muscle tissue form | Promtan et al. (2023) |
| | Cherry Valley White-crested Jianchang (Duck) | Pre-adipocytes cells | Fat tissue form | Wang et al. (2018) |
| | Turkey | Satellite cells | Muscle tissue form | Clark et al. (2016) |
| Mammalian | Sheep | Satellite cells | Muscle tissue form | Carpenter et al (2000) |
| | Goat | Muscle stem cells | Muscle tissue form | Sui et al. (2018) |
| | Horse | Mesenchymal stem | Chondrogenic tissue | Fülber et al. |

642 Table 3. Types of cell donors for manufacturing cultured meat

| | | cells | form | (2021) |
|---------|----------------------|-------------------------|--------------------|----------------------------|
| | Camel | Skin fibroblasts cells | Skin tissue form | Saadeldin et al. (2019) |
| | Deer | Mesenchymal stem cells | Muscle tissue form | Luo et al. (2022) |
| Fishery | Atlantic salmon | Adipose cells | Fat tissue form | Vegusdal et al. (2003) |
| | Large yellow croaker | Piscine satellite cells | Muscle tissue form | Zhang et al. (2023) |
| | Bluefin tuna | Cells | Tissue form | Bain et al. (2013) |
| | Greasyback shrimp | Cells | Tissue form | Zhao et al. (2023) |
| | Lobster | Primary muscle cells | Muscle tissue form | Jang et al. (2022) |
| | | | | |



645646 Figure 1. The whole process for manufacturing cultured meat.