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# **REVIEW** The Color-Developing Methods for Cultivated Meat and Meat Analogues: A Mini-Review

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**Abstract** Novel meat-inspired products, such as cell-cultivated meat analogues, embrace environmental sustainability, food safety and security, animal welfare, and human health, but consumers are still hesitant to accept these products. The appearance of food is often the most persuasive determinant of purchasing decisions for food. Producing cultivated meat and meat analogues with similar characteristics to conventional meat could lead to increased acceptability, marketability, and profitability. Color is one of the sensorial characteristics that can be improved using color-inducing methods and colorants. Synthetic colorants are cheap and stable, but natural pigments are regarded as safer components for novel food production. The complexity of identifying specific colorants to imitate both raw and cooked meat color lies in the differences in ingredients and methods used to produce meat alternatives. Research devoted to improving the sensorial characteristics of meat analogues has noted various color-inducing methods (e.g., ohmic cooking and pasteurization) and additives (e.g., lactoferrin, laccase, xylose, and pectin). Additionally, considerations toward other meat components, such as fat, can aid in mimicking conventional meat appearance. For instance, the use of plant-based fat replacers and scaffolds can produce a marked sensory enhancement without compromising the sustainability of alternative meats. Moving forward, consumer-relevant sensorial characteristics, such as taste and texture, should be prioritized alongside improving the coloration of meat alternatives.

Keywords cultured meat, meat analogue, colorant, plant-based meat, mycoprotein

# Introduction

Color is one of the main contributors to the overall palatability of food. Whether raw or cooked, the impact of color on consumer preference and purchase intention for food is highly expected. The psychological impact of food color is an important consideration for food manufacturers because it can affect the palatability and marketability of products (Spence, 2015). Thus, innovations and the development of food colorants will always be relevant in the food industry.

Meat color is primarily determined by the content and chemical state of myoglobin

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The unraveling of meat biochemistry has led to the gradual development of methods for assessing the sensorial acceptability (e.g., color, texture, and taste) of meat and meat products. Among these methods is the development of colorants aimed to retain or improve the color of meat and meat products. However, the discovery of the potential dangers of some, if not all, synthetic food colorants (e.g., Amaranth S, Ponceau, etc.), particularly their association with carcinogenic, genotoxic, and neurotoxic effects, has been noted (Kobylewski and Jacobson, 2012). Due to these food safety concerns, the call for safer alternatives has led to the sourcing of natural food colorants that offer stability, sustainability, and cost-effectiveness (Novais et al., 2022).

Cultivated meat and meat analogues are types of novel foods ideally improved using colorants to achieve a similar color to their conventional counterparts. Color changes during storage and market display could result in decreased consumer acceptability and, in turn, potential profit loss and food wastage. Therefore, methods for retaining or improving the color characteristics of meat analogues have been developed to resolve these issues (Ryu et al., 2023). The development of colorants and their applicability to novel foods could further improve product acceptability, leading to improved consumer experience and product profitability. This review focuses on the recent methods and colorants used for the color development and improvement of meat-inspired novel foods.

# **Novel Foods and Their Colors**

#### **Cultivated meat**

Cultivated meat is laboratory-grown meat produced by culturing muscle precursor cells and other meat-relevant cell types so that following their proliferation and differentiation processes, they are ultimately transformed into edible, sustainable, and ethically-sound meat. Currently, two companies (Upside Foods and Good Meat) have secured approval for the commercialization of cultured chicken meat (Mariano et al., 2023). Cultivated meat is classified and regulated under the "Novel Foods" standards worldwide, considering its nature of production (GFI APAC, 2023).

In terms of color, the lack of Mb in raw cultivated meats—a result of the suppression of Mb under ambient oxygen conditions—calls for techniques to improve its pale color to achieve a coloration near that of conventional meat types (Fraeye et al., 2020). Several strategies related to cultivated meat color improvement have been reported which either target color change during cell mass production or post-cell mass production. Color improvement of cultivated meat during cell mass production could include methods and/or materials that either directly or indirectly affect the visual impact of the raw outputs. Simsa et al. (2019) showed that the supplementation of Mb or hemoglobin (Hb) can improve the resulting color of raw and cooked cultured beef with prolonged incubation time. However, the use of animal-derived components, such as blood-derived proteins, either for coloration or as a source of growth factors, for cultivated meat production is discouraged (Reiss et al., 2021). Meanwhile, the possibility of generating color-enhanced meat products through cell culture by genetically engineering the endogenous expression of non-native carotenoids into primary bovine skeletal muscle cells was demonstrated (Stout and Kaplan, 2022). Additionally, Ong et al. (2021) harnessed the color-changing properties of jackfruit-based scaffolds for porcine myoblasts to imitate the characteristic meat browning of conventional meat while cooking. On the other hand, Yen et al.

(2023) mimicked meat marbling using fat replacers derived from plant-based oleogels, an example of post-production processing for cultivated meat.

Due to the infancy of cultivated meat technology, cultivated meat companies and other academic reports do not disclose specific information on the color development of cultured meat products brought about by patentability of technology and company confidentiality. Also, the prioritization of cell line establishment, development of mass production methods, improvement of scaffolding strategies, and development of serum-free media seems logical, considering that majority of the production cost is incurred during cell mass production. Given this point, the improvement of sensorial characteristics is reserved until efficient cell mass production is achieved.

#### **Meat analogues**

Meat analogues have been developed as an alternative to conventional meat and are generally categorized into plant-, insect- and microbial biomass-based, according to the main component of the products. Ultimately, meat analogues must provide meat-like sensorial characteristics but without conventional livestock meat. The attitude toward novel foods, generally rooted in the perception of protein sources, needs to be addressed by overcoming the challenges of increasing the acceptability of these products (Deroy et al., 2015; Schouteten et al., 2016). This section presents the recent developments in meat analogues, focusing on the currently reported technologies and colorants for product development.

#### **Plant-based**

There is a wide variety of plant proteins that can be used in the production of meat analogues. Common sources of these proteins include soybean, wheat, pea, and other high protein-producing plants that can be converted into food materials (Ahmad et al., 2022; Kumar et al., 2017). Although deemed as a healthier option, plant-derived components tend to impart a bitter, astringent, and beany flavor, which can lower the sensorial acceptability of meat analogues into which they are incorporated (Kumar et al., 2017; Wang et al., 2022). Thus, efforts are to be made to control these unfavorable characteristics.

Given that the goal of plant-based meat is to simulate conventional meat, the raw and cooked product color is another important consideration for improving overall acceptability. Raw plant-based meats often have a yellowish color, far from the conventional CIE a\* of conventional meat. Many commercial colorants, natural and synthetic, can be used to improve the color of plant-based meat. Impossible Food launched a plant-based beef patty with a nearly authentic color to conventional beef patties by incorporating heme proteins from genetically modified yeast (McDermott, 2021). However, taboos around genetic modifications tend to affect product perception and acceptability. For this reason, a plethora of research has ventured into discovering natural pigments for plant-based meat alternatives in line with the growing inclination for cheap and safe naturally sourced products. Bakhsh et al. (2023) recently showed different pigment (e.g.; anthocyanin, betalain, chlorophyl, etc.) sources (e.g., paprika, monascus, grape, red cabbage, etc.) that could be used for meat alternatives. Plant-based patty containing monascus red showed the highest CIE a\* score in raw (25.15±0.39), steamed (20.63±0.85), and cooked (19.26±1.60) categories and were significantly higher than the control beef patty. Meanwhile, paprika (10.42±0.45) was the nearest to the CIE a\* of cooked beef patty (10.20±1.29). Overall, the study explored multiple pigment sources which could aid in the formulation of meat analogues.

Currently, numerous plant-based meat companies have already commercialized their products. As a prerequisite for commercializing novel foods, labeling, which includes the components used in the production of the final product, such as natural and synthetic additives, provides the information necessary to assess its safety and truthfulness of claims. Common

among product labels is the declaration of coloring agents used which could guide consumers perception towards the product.

#### Insect-based

Entomophagy, the consumption of insects as part of the diet, has been practiced in most regions of the world even prior to the coining of the term (Evans et al., 2015). The increasing interest in consuming insects, whether processed or in actual form, continues to present opportunities to develop their overall characteristics as food to overcome the fear or disgust toward entomophagy (Clarkson et al., 2019). Technologies have been developed to transform insects into unrecognizable forms, such as flours and powders (Melgar-Lalanne et al., 2019). Additionally, bleaching pre-treatment for edible insects results in the removal of natural pigments of insects to resemble a white-to-yellowish color (Triunfo et al., 2022). Therefore, coloring pigments are needed to compensate for the objective meat color. However, research on the improvement of sensorial characteristics of insect-based meat analogues seems to be lacking compared to the numerous publications dedicated to plant-based meat production. This is evident in research and reports that make use of insect-based flours or powders as additives, not as the major component of meat analogues (Foreman, 2023; Neo, 2021). Jones (2023) reviewed that the production of insect powders is energy- and labor-intensive, resulting in greater price disparity compared to chicken, tofu, conventional beef, and vegetables. More importantly, insect farming still has welfare issues, which are mainly centered around the sentience and cognition of insects. However, future developments may use insect protein as a major component once scale-up of production and other challenges are addressed. Once reached, the improvement of sensorial characteristics, including coloration, of insect-based meat analogues could take place.

#### Mycoprotein-based

Another popular meat analogue is mycoprotein-based food. Edible fungi have been part of the human diet for time immemorial and have a long history of use as pharmaceuticals. Advancements in fermentation technology have allowed the biomass production of fungal mycelia. The biomass is then processed, which includes dewatering and drying, followed by adding binders, flavors, and colorants to achieve meat-like characteristics. A popular commercial mycoprotein-based meat analogue is based on the biomass of *Fusarium venenatum* (PTA-2684), which can be processed into meat-inspired products, including those that are manufactured from chicken or beef (Fellows, 2009; Wiebe, 2002). Mycoprotein pastes are often described as light in color. Therefore, the addition of spices and colorants is important to simulate the heme color of conventional meat (Hashempour-Baltork et al., 2023).

# **Potential Colorants for Novel Foods**

#### **Food colorants**

There is an array of food colorants commercially available for cultured meat and meat analogues owing to years of research to overcome meat color deterioration and improve the sensorial acceptability of meat-derived products. In this section, products and research related to natural and synthetic food colorants will be explored and highlighted to assist in the selection of colorants for meat-inspired novel foods, such as cultured meat and meat analogues.

#### Synthetic colorants

Studies on synthetic colorants in meat and meat products are more focused on their detection rather than exploration of

their practical use. The negative connotation of the application of synthetic colorants to meat products began with the discovery of their adverse effects on human and animal health when consumed at unsafe levels (Gupta et al., 2019; Kobylewski and Jacobson, 2012; Miller et al., 2022). However, food regulatory agencies still acknowledge and allow the use of synthetic colorants, as seen in the guidelines on the application levels and specific conditions of use (McAvoy, 2014). In the United States, the Federal Food, Drug, and Cosmetic Act (FD&C Act) guides the regulation of food dyes and colorants. While this Act has been standing, the stricter regulation of synthetic colorants compared to natural pigments somehow provides a disadvantage in using synthetic colorants, given that natural colorants are exempted from Food and Drug Administration (FDA) regulation (Simon et al., 2017).

Cultured meat and meat analogues undergo a series of physical and chemical changes as components are mixed and engineered to formulate and design a product with meat-like characteristics. During processing, raw materials (natural or synthetic) contribute to the changes (e.g., color, pH, and water holding capacity) that can affect the final characteristics of the product, particularly the color. Synthetic colorants are known to be cheap, light- and pH-resistant, and color-stable, which makes them suitable color additives for highly processed foods, such as meat alternatives. Some of the common synthetic colorants used in meat products include Amaranth S, Ponceau, Allura Red, Carmoisine, Erythrosine, and Sudan, which are regulated dyes due to their potential health adverse reactions (Iammarino et al., 2019). Potential synthetic colorants for cultured meat and meat analogues along with their acceptable daily intake (ADI) are the following: Carmoisine (ADI: 0–4 mg/kg), Ponceau 4R (ADI: 0.7 mg/kg), Erythrosine (ADI: 0–0.1 mg/kg), and Allura Red [ADI: 0–7 mg/kg; The European Food Safety Authority (EFSA) Panel on Food Additives and Nutrient Sources Added to Food, 2009b; EFSA Panel on Food Additives and Nutrient Sources Added to Food, 2009b; EFSA Panel on Food Additives and Nutrient Sources Added to Food, 2009b; EFSA Panel on Food Additives and Nutrient Sources Added to Food, 2009c; EFSA Panel on Food Additives and Nutrient Sources Added to Food, 2009b; EFSA Panel on Food Additives and Nutrient Sources Added to Food, 2009c; EFSA Panel on Food Additives and Nutrient Sources Added to Food, 2011]. However, the use of these synthetic colorants should be carefully considered with regard to consumer perception toward the inclusion of these colorants, which could affect the acceptability and safety of cultured meat analogues. Fig. 1 shows the result of surveying online shopping sites



Fig. 1. Pie chart of colorants used by plant-based meat companies with commercial meat analogues in Amazon and Plant X.

(Amazon and Plant X) selling plant-based meat analogues. A total of 49 meat analogue companies declared colorants in their product labels, but only eight companies revealed the inclusion of synthetic colorants, such as Titanium dioxide and Caramel color. Other synthetic colorants used include FD&C Red, Iron oxide, and Vitamin B12 (cyanocobalamin; Fig. 1). Although colorants used were identified, specific amounts of colorant used in the product were not disclosed.

#### Natural colorants

As an alternative to synthetic colorants, pigments from natural resources, mainly from plants, have been discovered and are still being developed. Unlike synthetic colorants, these natural pigments are exempt from the arduous process of dye certification as a food additive, given that plant-based dyes are commonly produced from edible plants rich in colored metabolites. Most food color sensory assessments are guided by CIE L\*, CIE a\*, and CIE b\* parameters using either a colorimeter or spectrometer as a source of empirical data, then followed by subjective assessments by consumers and/or professionals. Altogether, food coloration is systematically achieved via guided formulations based on empirical data and consumer preferences.

Commercially available meat analogues from diverse companies could be purchased either online or in markets. The availability of product labels provides the opportunity for consumers to scrutinize the products in advance. As shown in Fig. 1, the majority of commercial plant-based meat uses plant-sourced colorants, including paprika, tomato (paste or powder), beet juice, and lycopene, with paprika being the most used colorant. Some products vaguely declared colorants as fruit extracts, fruit and vegetable juice, and vegetable juices, while other natural colorants include red rice yeast, red miso paste, red radish, soy leghemoglobin, annatto, turmeric, and carrageenan, directed to imitate the CIE a\* of meat and meat products. However, the amount of the specific colorants used among the products was not disclosed. Therefore, imitating the CIE a\* of these products could be based on experiments to determine the optimal amount of colorant to be used.

Accordingly, research has been published to discover methods and colorants to improve the sensorial characteristics of meat analogues. Table 1 shows recent research that includes color as a parameter in improving meat analogues. Overall, most of the colorants are natural pigments dominated by the use of beets or paprika. Other techniques used to induce coloration include the addition of plant-based replacers, heme proteins (Hb and Mb), decompartmentalization, *Haematococcus pluvialis*, ohmic cooking, and color-enhancing additives, such as xylose, laccase, pectin, and lactoferrin (Bakhsh et al., 2022; Huang et al., 2024; Jung et al., 2022; Liu et al., 2023; Ong et al., 2021; Sakai et al., 2022; Simsa et al., 2019; Wen et al., 2022; Xia et al., 2022; Yen et al., 2023). Although the usual color of focus for meat analogues is meat-like red, the cooked coloration was also given importance.

In cell-cultivated meat, culturing bovine satellite cells with Mb showed increased proliferation and development of cooked color similar to cooked beef after cooking (Simsa et al., 2019). Furthermore, the innovative use of jackfruit-based scaffolds both imitated meat marbling and resembled the browning of meat when oxidized (Ong et al., 2021). Additionally, Wen et al. (2022) designed three-dimensional-printed meat analogues containing xylose and beet extract to imitate the color change of conventional meat.

Research on plant-based meat analogue development has determined specific or recommended amounts of natural pigments in meat analogues to achieve conventional meat-like coloration. Lyu et al. (2023) showed that the addition of 10% (w/w) tomato peel powder could provide color-changing behavior similar to conventional meat. Similarly, Sakai et al. (2022) studied the combination of laccase, pectin, and beet red pigment to simulate the color change of meat. Another study identified that the combination of cacao (1.1–1.3 mg/g) and red beet (0.4–1.5 mg/g) could produce a similar coloration to a

Meat alternative category	Research	Colorant/Method used	Results	References
Cell-cultivated meat	Cultured meat platform developed through the structuring of edible microcarrier-derived microtissues with oleogel- based fat substitute	Plant-based fat replacer	<ul> <li>Darker color of raw cultured meat prototypes compared to raw beef was observed</li> <li>Cooking resulted in further darkening attributed to the Maillard reaction</li> </ul>	Adapted from Yen et al. (2023) with CC-BY.
	Extracellular heme proteins influence bovine myosatellite cell proliferation and the color of cell-based meat	Heme protein	<ul> <li>An increase in pigment content of bovine satellite cells was noticed when cultured with heme proteins (i.e., Hb and Mb)</li> <li>BSC grown with Mb showed the closest similarity to cooked beef</li> </ul>	Adapted from Simsa et al. (2019) with CC-BY.
			Norm         Norm           By	
	Decompartmentalization as a simple color manipulation of plant-based marbling meat alternatives	Decompartmentalization and jackfruit-based scaffold	<ul> <li>The color-changing behavior of the scaffold resembled meat-like browning</li> <li>Polyphenol-based color was sensitive to pH change (i.e., pH 2 and 11).</li> </ul>	Adapted from Ong et al. (2021) with permission of Elsevier.
			a         by 5 by 1 by 2 by 1 by 3 by 3	
Plant-based	Effect of xylose on rheological, printing, color, texture, and microstructure characteristics of 3D-printable colorant- containing meat analogs based on mung bean protein	Xylose and beet red	<ul> <li>Addition of approximately 0.5%–2% w/w beet root could mimic chicken, pork, and beef color</li> <li>Xylose addition could affect the texture and color due to the Maillard reaction</li> </ul>	Adapted from Wen et al. (2022) with permission of Elsevier.
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Meat alternative category	Research	Colorant/Method used	Results	References
Plant-based	Effect of whole tomato powder or tomato peel powder incorporation on the color, nutritional, and textural properties of extruded high- moisture meat analogues	Tomato powder and tomato peel powder	<ul> <li>Color change in meat analogue containing 10% w/w tomato peel powder could resemble the color change in cooking real meat</li> <li>Addition of whole tomato powder in meat analogues could resemble cured- meat products</li> </ul>	Adapted from Lyu et al. (2023) with CC-BY.
			Ai) Ab) A'a) A'b) Control Bay By WTP5 Ca) Cby C'a) C'b)	
			WIP10 Boiled WTP10 Da) Db) WTP20 La) Eb) E'a) E'b) E'b)	
			TPP10 Roited TPP10	
	Effects of <i>Haematococcus</i> <i>pluvialis</i> addition on the sensory properties of plant- based meat analogues	H. pluvialis	- Addition of less than 5% of <i>H. pluvialis</i> can improve the color of plant-based meat to beef-like red	Adapted from Liu et al. (2023) with CC-BY.
	Structural and rheological properties of meat analogues from <i>H. pluvialis</i> residue-pea protein by high-moisture extrusion	H. pluvialis	<ul> <li>The addition of 10–40 g/100 g of <i>H. pluvialis</i> in meat analogue can induce coloration similar to dried red meat</li> <li>Treatments with <i>H. pluvialis</i> also improved the texture after high-moisture extrusion</li> </ul>	Xia et al. (2022)
	Utilizing <i>H. pluvialis</i> to simulate animal meat color in high-moisture meat analogues: Texture quality and color stability	H. pluvialis	<ul> <li>Treatments with 1% or 0.25% <i>H. pluvialis</i> resembled raw beef loin and raw pork loin, respectively</li> <li>Color loss was greater due to light exposure than to cooking and frozen storage</li> </ul>	Adapted from Huang et al. (2024) with CC-BY.
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Meat alternative category	Research	Colorant/Method used	Results	References
Plant-based	Synergistic effects of laccase and pectin on the color changes and functional properties of meat analogues containing beet red pigment	Beet red, laccase, and sugar beet pectin	<ul> <li>The combination of laccase and pectin beet red-containing plant-based patties resulted in a similar grilled beef patty color</li> <li>Laccase and sugar beet pectin can be utilized to improve the browning system of meat analogues containing beet red</li> </ul>	Adapted from Sakai et al. (2022) with CC-BY.
			Before griling (Front)     Image: Constraint of the second s	
			10 mm Non treated patty LC-treated patty LC-treated patty LC-treated patty with BR + MC with BR + MC with BR + SBP BR + MC + SBP (Control)	
	Beetroot juices as colorant in plant-based minced meat analogues: Color, betalain composition, and antioxidant activity as affected by juice type	Beetroot juices	<ul> <li>Addition of cooked beet root juice in textured soy protein could mimic raw minced beef color</li> <li>The combination of commercial beet juice and textured soy protein could resemble raw minced pork color</li> </ul>	Adapted from Fernández-López et al. (2023) with CC-BY- NC-ND.
			CBJ (1:4) + TPSOY CKBJ (1:3) + TPSOY	
	Application of obmic cooking	Ohmic cooking and	PORK MINCED MEAT BEEF MINCED MEAT	Jung et al
	to produce a soy protein-based meat analogue	beet red	induces brighter internal coloration of meat analogue	(2022)
	Varying the amount of solid fat in animal fat mimetics for plant-based salami analogues influences texture, appearance, and sensory characteristics	Plant-based fat from canola, sal, and beetroot powder	<ul> <li>Addition of 25%–50% sal fat in meat analogue could mimic pork fat even after drying</li> <li>Pasteurization induces a color change of the product from deep red to orange-red</li> </ul>	Dreher et al. (2021)
	Physicochemical properties of novel non-meat sausages containing natural colorants and preservatives	Lycopene, paprika oleoresin, and red yeast rice powder	- Superior sensory acceptance was observed for non-meat sausages containing paprika oleoresin (3 g/kg), red yeast rice (0.1 g/kg), and lycopene (0.32 g/kg)	Akramzadeh et al. (2018)

Meat alternative category	Research	Colorant/Method used	Results	References
Plant-based	Development of plant-based burgers using gelled emulsions as fat source and beetroot juice as colorant: Effects on chemical, physicochemical, appearance, and sensory characteristics	Beetroot juice	<text></text>	Adapted from Botella-Martínez et al. (2022) with CC-BY-NC-ND.
	Synergistic effect of lactoferrin and red yeast rice on the quality characteristics of novel plant-based meat analog patties	Lactoferrin and red yeast rice powder	Raw treatment patties were lighter with moderate CIE b* compared to raw beef patties	Adapted from Bakhsh et al. (2022) with CC-BY.
	Applications of various natural pigments to a plant-based meat analogue	Beet red, <i>Monascus</i> red, - paprika oleoresin, sorghum, and cacao	<ul> <li>The combination of cacao (1.1–1.3 mg/g) and red beet (0.4–1.5 mg/g) showed similar coloration to Hanwoo rib patty</li> <li>Single colorant in meat analogue was insufficient in mimicking the control</li> </ul>	Adapted from Ryu et al. (2023) CC-BY-NC-ND.
	Characterization of plant-based meat alternatives blended with anthocyanins, chlorophyll, and various edible natural pigments	Paprika, <i>Monascus</i> , grape, cherry, red cabbage, and beet red	<ul> <li>Plant-based meat analogues with natural pigments showed lower CIE a* compared to conventional meat</li> <li>Inconsistencies in the color coordinates of the treatments may be due to differences in the color of natural pigments, concentration, and substitution of plantbased protein</li> <li>Image: Concentration of the substitution of plantbased protein</li> </ul>	Adapted from Bakhsh et al. (2023) with CC-BY.

Meat alternative category	Research	Colorant/Method used	Results	References
Plant-based	Optimizing the appearance of plant-based foods using color match theory	Turmeric, beet red, butterfly pea flower	- More than three pigments should be used to mimic animal-based products	Wannasin et al. (2023)
Mycoprotein -	Meat substitute development from fungal protein (Aspergillus oryzae)	Beet extract and annatto	<ul> <li>Beet extract closely resembled real meat color</li> <li>Annato showed higher saturation and CIE a* intensity, giving an artificial or unnatural appearance</li> </ul>	Gamarra-Castillo et al. (2022)
	Mushroom–legume-based minced meat: Physico- chemical and sensory properties	Beet root extract	- Beet root extract can enhance mushroom-based minced meat substitutes and achieve a high sensory acceptance at 0.2% (w/w)	Mazumder et al. (2023)
	Mycoprotein as chicken meat substitute in nugget formulation: Physicochemical and sensorial characterization	Carrageenan	<ul> <li>The use of mycoprotein as chicken substitute increased the CIE a* of the product</li> <li>The addition of carrageenan did not affect the CIE a* of the mycoprotein- based nuggets</li> <li>White pepper, instead of black pepper, can be used to increase the CIE L*</li> </ul>	Hashempour- Baltork et al. (2023)

Research papers pooled from Google Scholar using keywords: "colorant", "meat analogue", "cultivated", "plant-based", and "mycoprotein". Hb, hemoglobin; Mb, myoglobin; BSC, bovine satellite cells.

Hanwoo rib patty (Ryu et al., 2023). However, Ryu et al. (2023) concluded that the use of a single colorant for meat analogues is not enough to mimic real meat color, and Wannasin et al. (2023) concluded that more than three pigments should be used with the aid of color match theory. Aside from terrestrial plant sources of red pigments, researchers have explored the use of microalgae, such as *H. pluvialis*, as a colorant for meat analogues. According to Liu et al. (2023), the addition of less than 5% (w/w) of *H. pluvialis* was enough to resemble beef-like red, which was similar to the reported amount of 0.25% or 1% (w/w) to imitate raw pork loin and raw beef loin, respectively (Huang et al., 2024). A relatively greater amount of 10%–40% (w/w) of *H. pluvialis* residue imitated the color of dried red meat (Xia et al., 2022). Additionally, Akramzadeh et al. (2018) formulated meat analogues containing carrageenan, modified cornstarch, egg white, and natural colorants (lycopene, paprika oleoresin, and red yeast rice powder) and optimized the levels of all these ingredients to yield a product with the highest overall acceptability. Bakhsh et al. (2022) also used red yeast rice as a source of color for plant-based meat.

Similar to cultivated meat, research related to mycoprotein-based analogues is limited in terms of studies involving color parameters. Gamarra-Castillo et al. (2022) compared the effect of beet extract and annatto, which resulted in the preference for beet root extract because the use of annatto could result in an artificial appearance. Beet root extract (0.2%, w/w) enhanced the color of a mushroom-based minced meat substitute, both fresh and cooked (Mazumder et al., 2023). Contrary to the need for colorants, Hashempour-Baltork et al. (2023) reported that using mycoprotein increased the CIE a\* of chicken nuggets formulated with carrageenan. Moreover, it was suggested that white pepper can be used to modulate the CIE L\* of the product.

Despite the focus on achieving meat-like red for meat analogues, other research focused on fat alternatives, which also contribute to the color and overall appearance of both conventional meat and meat analogues. Yen et al. (2023) developed an

oleogel-based fat replacer for cultivated meat. Meanwhile, Dreher et al. (2021) focused on developing fat replacers from canola and sal. They found that the addition of 25%–50 % w/w of sal fat satisfactorily mimicked pork fat in raw and dried meat analogues. Aside from colorants, a high ohmic cooking temperature can induce improved internal coloration of meat analogues (Jung et al., 2022). Additionally, pasteurization could induce a color change in meat analogues, as observed in meat analogues with beetroot powder, which transitioned from deep red to orange-red after pasteurization (Dreher et al., 2021).

Although the addition of natural pigments is directed toward improving the sensorial characteristics of meat alternatives, the potential bioactivity of these additives should also be considered. Fernández-López et al. (2023) compared the antioxidant activities of different beetroot juices and identified that fresh-cooked beetroot juice yielded the highest antioxidant activity, attributed to the high betalain content of freshly extracted beet juice compared to commercial ones. Jin et al. (2021) revealed that pigments sourced from blueberries, cherries, onions, black tea, and clove buds had higher antioxidant activity than other plant-derived pigments and conventional meat. However, the stability of these bioactive components during meat analogue production and after cooking must be evaluated before the product can be considered a functional food.

# Conclusion

The increasing interest in meat alternatives continues to engage the industry sector and researchers in the development of cultured meat and meat analogues that mimic conventional meat characteristics, including meat color. Diverse techniques can be used to achieve the desired color of meat alternatives. The use of synthetic and natural colorants presents an affordable and efficient option to modulate the color characteristics of cultured meat and meat analogues. However, the potential adverse effects of these components on human health must be considered during the formulation of food products. Thus, other color-inducing methods, such as the use of color-changing scaffolds, ohmic cooking, and pasteurization, could be used in tandem with the colorants. Furthermore, attention toward mimicking other meat components, such as fat, could improve the overall appearance of cultured meat and meat analogues and potentially increase product acceptability. Therefore, the optimization of color-inducing techniques can contribute to increasing the overall acceptability of novel meat-inspired foods.

# **Conflicts of Interest**

The authors declare no potential conflicts of interest.

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# **Author Contributions**

Conceptualization: Mariano E Jr, Hur SJ. Investigation: Mariano E Jr, Lee DY, Yun SH, Lee J, Choi Y, Park J, Han D, Kim JS. Writing - original draft: Mariano E Jr, Hur SJ. Writing - review & editing: Mariano E Jr, Lee DY, Yun SH, Lee J, Choi Y,

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# **Ethics Approval**

This article does not require IRB/IACUC approval because there are no human and animal participants.

## References

- Ahmad M, Qureshi S, Akbar MH, Siddiqui SA, Gani A, Mushtaq M, Hassan I, Dhull SB. 2022. Plant-based meat alternatives: Compositional analysis, current development and challenges. Appl Food Res 2:100154.
- Akramzadeh N, Hosseini H, Pilevar Z, Khosroshahi NK, Khosravi-Darani K, Komeyli R, Barba FJ, Pugliese A, Poojary MM, Khaneghah AM. 2018. Physicochemical properties of novel non-meat sausages containing natural colorants and preservatives. J Food Process Preserv 42:e13660.
- Bakhsh A, Cho C, Baritugo KA, Kim B, Ullah Q, Rahman A, Park S. 2023. Characterization of plant-based meat alternatives blended with anthocyanins, chlorophyll, and various edible natural pigments. Int J Food Prop 26:1546-1565.
- Bakhsh A, Lee EY, Bakry AM, Rathnayake D, Son YM, Kim SW, Hwang YH, Joo ST. 2022. Synergistic effect of lactoferrin and red yeast rice on the quality characteristics of novel plant-based meat analog patties. LWT-Food Sci Technol 171: 114095.
- Botella-Martínez C, Viuda-Martos M, Fernández-López JA, Pérez-Alvarez JA, Fernández-López J. 2022. Development of plant-based burgers using gelled emulsions as fat source and beetroot juice as colorant: Effects on chemical, physicochemical, appearance and sensory characteristics. LWT-Food Sci Technol 172:114193.
- Clarkson C, Birch J, Mirosa M. 2019. Locusts as a source of lipids and proteins and consumer acceptance. In Encyclopedia of food chemistry. Melton L, Shahidi F, Varelis P (ed). Elsevier, Amsterdam, Netherlands. pp 167-172.
- Deroy O, Reade B, Spence C. 2015. The insectivore's dilemma, and how to take the West out of it. Food Qual Prefer 44:44-55.
- Dreher J, König M, Herrmann K, Terjung N, Gibis M, Weiss J. 2021. Varying the amount of solid fat in animal fat mimetics for plant-based salami analogues influences texture, appearance and sensory characteristics. LWT-Food Sci Technol 143:111140.
- EFSA Panel on Food Additives and Nutrient Sources Added to Food. 2009a. Scientific opinion on the re-evaluation of azorubine/carmoisine (E 122) as a food additive. EFSA J 7:1332.
- EFSA Panel on Food Additives and Nutrient Sources Added to Food. 2009b. Scientific opinion on the re-evaluation of Ponceau 4R (E 124) as a food additive. EFSA J 7:1328.
- EFSA Panel on Food Additives and Nutrient Sources Added to Food. 2009c. Scientific opinion on the re-evaluation of Allura Red AC (E 129) as a food additive. EFSA J 7:1327.
- EFSA Panel on Food Additives and Nutrient Sources Added to Food. 2011. Scientific opinion on the re-evaluation of erythrosine (E 127) as a food additive. EFSA J 9:1854.
- Evans J, Alemu MH, Flore R, Frøst MB, Halloran A, Jensen AB, Maciel-Vergara G, Meyer-Rochow VB, Münke-Svendsen C, Olsen SB, Payne C, Roos N, Rozin P, Tan HSG, van Huis A, Vantomme P, Eilenberg J. 2015. 'Entomophagy': An evolving terminology in need of review. J Insects Food Feed 1:293-305.
- Fellows PJ. 2009. Food biotechnology. In Food processing technology: Principles and practice. 3rd ed. Fellows PJ (ed).

Woodhead, Cambridge, UK. pp 229-270.

- Fernández-López J, Ponce-Martínez AJ, Rodríguez-Párraga J, Solivella-Poveda AM, Fernández-López JA, Viuda-Martos M, Pérez-Alvarez JA. 2023. Beetroot juices as colorant in plant-based minced meat analogues: Color, betalain composition and antioxidant activity as affected by juice type. Food Biosci 56:103156.
- Foreman P. 2023. Lidl launches insect burgers made with mealworm larvae. Available from: https://plantbasednews.org/ news/alternative-protein/lidl-insect-burgers/. Accessed at Oct 25, 2023.
- 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.
- Gamarra-Castillo O, Echeverry-Montaña N, Marbello-Santrich A, Hernández-Carrión M, Restrepo S. 2022. Meat substitute development from fungal protein (*Aspergillus oryzae*). Foods 11:2940.
- Gupta R, Ranjan S, Yadav A, Verma B, Malhotra K, Madan M, Chopra O, Jain S, Gupta S, Joshi A, Bhasin C, Mudgal P. 2019. Toxic effects of food colorants erythrosine and tartrazine on zebrafish embryo development. Curr Res Nutr Food Sci 7:876-885.
- Hashempour-Baltork F, Jannat B, Dadgarnejad M, Alizadeh AM, Khosravi-Darani K, Hosseini H. 2023. Mycoprotein as chicken meat substitute in nugget formulation: Physicochemical and sensorial characterization. Food Sci Nutr 11:4289-4295.
- Huang Z, Liu Y, An H, Kovacs Z, Abddollahi M, Sun Z, Zhang G, Li C. 2024. Utilizing *Haematococcus pluvialis* to simulate animal meat color in high-moisture meat analogues: Texture quality and color stability. Food Res Int 175:113685.
- Iammarino M, Mentana A, Centonze D, Palermo C, Mangiacotti M, Chiaravalle AE. 2019. Dye use in fresh meat preparations and meat products: A survey by a validated method based on HPLC-UV-diode array detection as a contribution to risk assessment. Int J Food Sci Technol 55:1126-1135.
- Jin S, Kim, G, Jeong J. 2021. Evaluation of the effect of inhibiting lipid oxidiation of natural plant sources in a meat model system. J Food Qual 2021:6636335.
- Jones N. 2023. Fungi bacon and insect burgers: A guide to the proteins of the future. Nature 619:26-28.
- Jung AH, Hwang JH, Jun S, Park SH. 2022. Application of ohmic cooking to produce a soy protein-based meat analogue. LWT-Food Sci Technol 160:113271.
- Kobylewski S, Jacobson MF. 2012. Toxicology of food dyes. Int J Occup Environ Health 18:220-246.
- Kumar P, Chatli MK, Mehta N, Singh P, Malav OP, Verma AK. 2017. Meat analogues: Health promising sustainable meat substitutes. Crit Rev Food Sci Nutr 57:923-932.
- Liu M, Wang Y, Zhu L, Zhao X. 2023. Effects of *Haematococcus pluvialis* addition on the sensory properties of plant-based meat analogues. Foods 12:3435.
- Lyu X, Ying D, Zhang P, Fang Z. 2023. Effect of whole tomato powder or tomato peel powder incorporation on the color, nutritional, and textural properties of extruded high moisture meat analogues. Food Bioprocess Technol 17:231-244.
- Mancini RA, Hunt MC. 2005. Current research in meat color. Meat Sci 71:100-121.
- Mariano EJ, Lee DY, Yun SH, Lee J, Lee SY, Hur SJ. 2023. Checkmeat: A review on the applicability of conventional meat authentication techniques to cultured meat. Food Sci Anim Resour 43:1055-1066.
- Mazumder MAR, Sukchot S, Phonphimai P, Ketnawa S, Chaijan M, Grossmann L, Rawdkuen S. 2023. Mushroom–legumebased minced meat: Physico-chemical and sensory properties. Foods 12:2094.
- McAvoy SA. 2014. Global regulations of food colors: The manufacturing confectioner. Available from: https://www.

iacmcolor.org/wp-content/uploads/2014/09/PMCA-2014-McAvoy.pdf. Accessed at Oct 25, 2023.

- McDermott A. 2021. Looking to "junk" food to design healthier options. Proc Natl Acad Sci USA 118:e2116665118.
- Melgar-Lalanne G, Hernández-Álvarez AJ, Salinas-Castro A. 2019. Edible insects processing: Traditional and innovative technologies. Compr Rev Food Sci Food Saf 18:1166-1191.
- Miller MD, Steinmaus C, Golub MS, Castorina R, Thilakartne R, Bradman A, Marty MA. 2022. Potential impacts of synthetic food dyes on activity and attention in children: A review of the human and animal evidence. Environ Health 21:45.
- Neo P. 2021. Bug burger aspirations: Ento looks to emulate Beyond Meat success with launch of first insect burger patty. Available from: https://www.foodnavigator-asia.com/Article/2021/01/21/Bug-burger-aspirations-Ento-looks-to-emulate-Beyond-Meat-success-with-launch-of-first-insect-burger-patty. Accessed at Oct 25, 2023.
- Novais C, Molina AK, Abreu RMV, Santo-Buelga C, Ferreira ICFR, Pereira C, Barros L. 2022. Natural food colorants and preservatives: A review, a demand, and a challenge. J Agric Food Chem 70:2789-2805.
- Ong S, Loo L, Pang M, Tan R, Teng Y, Lou X, Chin SK, Naik MY, Yu H. 2021. Decompartmentalisation as a simple color manipulation of plant-based marbling meat alternatives. Biomaterials 277:121107.
- Reiss J, Robertson S, Suzuki M. 2021. Cell sources for cultivated meat: Applications and considerations throughout the production workflow. Int J Mol Sci 22:7513.
- Ryu KK, Kang YK, Jeong EW, Baek Y, Lee KY, Lee HG. 2023. Applications of various natural pigments to a plant-based meat analog. LWT-Food Sci Technol 174:114431.
- Sakai K, Sato Y, Okada M, Yamaguchi S. 2022. Synergistic effects of laccase and pectin on the color changes and functional properties of meat analogs containing beet red pigment. Sci Rep 12:1168.
- Schouteten JJ, De Steur H, De Pelsmaeker S, Lagast S, Juvinal JG, De Bourdeaudhuij I, Verbeke W, Gellynck X. 2016. Emotional and sensory profiling of insect-, plant- and meat-based burgers under blind, expected and informed conditions. Food Qual Prefer 52:27-31.
- Simon JE, Decker EA, Ferruzzi MG, Giusti MM, Mejia CD, Goldschmidt M, Talcott ST. 2017. Establishing standards on colors from natural sources. J Food Sci 82:2539-2553.
- Simsa R, Yuen J, Stout A, Rubio N, Fogelstrand P, Kaplan DL. 2019. Extracellular heme proteins influence bovine myosatellite cell proliferation and the color of cell-based meat. Foods 8:521.
- Spence C. 2015. On the psychological impact of food colour. Flavour 4:21.
- Stout A, Kaplan DL. 2022. Cultured meat product with genetically modified cells. US Patent App 17/615,578.
- Suman SP, Joseph P. 2013. Myoglobin chemistry and meat color. Annu Rev Food Sci Technol 4:79-99.
- The Good Food Institute Asia Pacific [GFI APAC]. 2023. Novel food regulations around the world. Available from: https://gfi-apac.org/novel-food-regulations-around-the-world/. Accessed at Oct 25, 2023.
- Triunfo M, Tafi E, Guarnieri A, Salvia R, Scieuzo C, Hahn T, Zibek S, Gagliardini A, Panariello L, Coltelli MB, De Bonis A, Falabella P. 2022. Characterization of chitin and chitosan derived from *Hermetia illucens*, a further step in a circular economy process. Sci Rep 12:6613.
- Wang Y, Tuccillo F, Lampi AM, Knaapila A, Pulkkinen M, Kariluoto S, Coda R, Edelmann M, Jouppila K, Sandell M, Piironen V, Katina K. 2022. Flavor challenges in extruded plant-based meat alternatives: A review. Compr Rev Food Sci Food Saf 21:2898-2929.
- Wannasin D, Grossmann LK, McClements DJ. 2023. Optimizing the appearance of plant-based foods using natural pigments

and color matching theory. Food Biophys 19:120-130.

- Wen Y, Kim HW, Park HJ. 2022. Effect of xylose on rheological, printing, color, texture, and microstructure characteristics of 3D-printable colorant-containing meat analogs based on mung bean protein. Food Res Int 160:111704.
- Wiebe M. 2002. Myco-protein from *Fusarium venenatum*: A well-established product for human consumption. Appl Microbiol Biotechnol 58:421-427.
- Xia S, Xue Y, Xue C, Jiang X, Li J. 2022. Structural and rheological properties of meat analogues from *Haematococcus pluvialis* residue-pea protein by high moisture extrusion. LWT-Food Sci Technol 154:112756.
- Yen FC, Glusac J, Levi S, Zernov A, Baruch L, Davidovich-Pinhas M, Fishman A, Machluf M. 2023. Cultured meat platform developed through the structuring of edible microcarrier-derived microtissues with oleogel-based fat substitute. Nat Commun 14:2942.