

Review article

Meat Analog Processing Technology from Non-animal Protein to Create Fibrous Structures Similar to Animal Meat: Review**Muhammad Izzuddin Noor Rahmadi¹, Tjahja Muhandri², Dase Hunaefi² and Raden Haryo Bimo Setiarto^{3,4*}***¹Food Science Program Study, Graduate School, IPB University, Dramaga, Bogor, West Java 16880, Indonesia**²Department of Food Science and Technology, Faculty of Agricultural Technology, and SEAFast Center - IPB University, Dramaga, Bogor, West Java 16880, Indonesia**³Research Center for Applied Microbiology, National Research and Innovation Agency (BRIN), Jalan Raya Jakarta-Bogor Km 46, KST Soekarno, Cibinong, Bogor, West Java 16911, Indonesia**⁴Research Collaboration Center for Traditional Fermentation, National Research and Innovation Agency (BRIN), Jl. Raya Jakarta-Bogor KM. 46, KST Soekarno, Cibinong, Bogor 16911, Indonesia*

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Abstract

Meat analogs or animal meat substitutes have become a significant alternative in the food industry, especially to meet consumer needs for healthy food products (cholesterol-free and low in saturated fatty acids) and to reduce the impact of environmental, ethical, religious, and health problems due to the excessive consumption of animal meat. One of the advantages of meat analog is that it can be formulated in such a way that its nutritional value is better than animal meat. However, meat analogs can have disadvantages, such as the product may lack the desired texture of meat. One solution to achieve a fibrous texture resembling animal meat can be done with a bottom-up technology approach and top-down technology. Protein sources in the manufacture of meat analog are classified into several categories, and one of the basic ingredients used is single cell mycoprotein. Mycoprotein, as an alternative protein source in the manufacture of meat analog, not only has a good nutritional profile that includes essential amino acids but it can also create a fibrous structure resembling animal meat. This study reviews the latest scientific reports on alternative protein sources from single cell mycoprotein, the nutritional value of single cell mycoprotein, meat analog processing technology and the potential and challenges associated with the development of meat analog in the future. Meat analog processing based on certain technologies are explained in this review.

Keywords: meat analog; single cell mycoprotein; processing technology; bottom-up technology; top-down technology

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1. Introduction

Beef is one of the foodstuffs favoured by consumers; this is because beef contains protein, carbohydrates, fat, and various types of minerals such as iron and phosphorus, which are good for health if consumed in moderation. According to the World Health Organization (WHO, 2023), the maximum consumption limit for red meat, such as beef, is 500 grams per week or equivalent to 70 g per day. The increasing interest in beef has caused the average value of beef consumption to increase over time. Based on data from the Ministry of Agriculture (Saragih 2023), the average level of beef consumption in the world in 2023 reached 6.4 kg per capita per year. In Indonesia, average beef consumption tended to increase over the period of 2013 to 2017, from 0.005 to 0.009 kg per capita per week, and then stagnated in 2021. The average beef consumption in Indonesia then increased again in 2022 to reach 0.010 kg per capita per week. The world's largest beef producer is the United States, which produced around 12,379,000 metric tons of beef in 2023, which accounted for 20.44% of world beef production. Indonesia is known to have produced around 379,703 tons of beef in 2021, with a total beef requirement of 664,286 tons. Excessive beef consumption triggers various aspects issues, such as the environment, ethics, religion, and health (Joseph et al., 2020).

According to the FAO (Food and Agricultural Organization) report submitted by Gerbens-Leenes *et al.* (2013), the livestock sector triggers climate change due to significant greenhouse gas emissions of around 14.5%, namely carbon dioxide, methane, and nitrous oxide. The impact of increasing beef production also causes intensive use of resources such as water and sufficient land, so large land clearing is often considered to encourage deforestation, cause pollution, and threaten the existence of biodiversity. The continued reliance on the livestock sector to meet the need for beef has a lot of negative impactson the environment (Gerbens-Leenes et al., 2013). Moreover, excessive demand for beef consumption has been found to be the cause of unethical handling of animals and excessive use of antibiotics in livestock (Joseph et al., 2020). For the religious aspect, the consumption of beef is prohibited for Hindus because of the belief that cows are considered a respected symbol of love, and the worship of cows is a form of Hindu religious value (Devi et al., 2014).

Besides these environmental, ethical, and religious issues, excessive beef consumption also triggers problems for human health. Excessive consumption of beef, with its high saturated fatty acid content and cholesterol, also increases LDL (low-density lipoprotein) levels in the blood. This increase in LDL in the blood can cause plaque buildup in the arteries, which can trigger atherosclerosis, heart disease, and stroke (Smith et al., 2020). According to the World Health Organization (WHO, 2023), excessive beef consumption is classified as a group 2A carcinogen, which is possibly carcinogenic to humans. This group includes hazardous substances such as nitrosamines that can be formed when meat is cooked (fried or grilled) at high temperatures (Feskens et al., 2013). The presence of several components in beef can cause health problems in humans due to excessive consumption, such as saturated fat, which increases LDL cholesterol levels; high cholesterol, which can increase blood cholesterol levels and contributes to cardiovascular disease, and heterocyclic amines (HCA) and polycyclic aromatic hydrocarbons (PAH), both of which form when beef is cooked at high temperatures (grilled or roasted) and are carcinogenic which can increase the risk of cancer. The existence of problems from various aspects due to excessive beef consumption can be replaced by consuming foods that are rich in fiber and low in fat through alternative protein sources. Alternative protein sources can be found in plants, insects, or microorganisms. The use of alternative protein sources

to replace animal meat is expected to reduce the negative impact on the environment by up to 50% and produce significant ecological sustainability. An alternative source of vegetable protein that can replace beef while still offering the nutritional equivalent to beef is meat analog (Ramachandraiah, 2021).

Meat analog is an alternative substitute for animal meat made from non-animal protein with physical and chemical characteristics similar to real meat (Kumar et al., 2017). It can be made from vegetable protein, which offers advantages such as lower calorie content and saturated fatty acids compared to animal meat (Sha & Xiong, 2020). Based on the protein source, meat analog can be made from several types of protein as basic ingredients, such as single cell protein (Ritala et al., 2017), plant protein (Yuliarti et al., 2021), and insect protein (Smetana et al., 2018). Single cell proteins such as algae, fungi, yeast, and bacteria are proteins that are efficiently produced because these microorganisms can be grown in a controlled environment and produce high biomass in a short time (Ritala et al., 2017). Vegetable proteins from plant materials such as soybeans, jack beans, and others are commercially available and are a source of protein that is easily obtained, cheap, and in demand by vegetarians and vegans who avoid consuming beef due to various negative aspects (Joseph et al., 2020). Protein from insects is also considered a sustainable protein source based on production efficiency and nutritional value that can be found in crickets, grasshoppers, and caterpillars (Stoops et al., 2017).



Figure 1. Meat analog from plant-based meat (Source: self-documentation)

Single cell mycoprotein, produced from fungi as an alternative protein source in the manufacture of meat analog, is of interest to the food industry. This is because mycoprotein produced during the fermentation process of the microorganism *Fusarium venenatum* provides a natural fibrous structure with low-fat content and no cholesterol (Caporgno et al., 2020). According to Molfetta et al. (2022), during the fermentation process by the microorganism *F. venenatum* for the manufacture of meat analog, protein fibers resembling animal meat are produced. The protein provides a texture similar to animal meat when compared to other vegetable proteins. Mycoprotein also has a high dietary fiber content and low fat content, making it a promising alternative source of protein for the manufacture of meat analog. Other research related to the manufacture of meat analog using *F. venenatum* mycoprotein found that the nutritional content was of high quality, with protein ranging from 44-50% of the total dry weight of biomass, insoluble fiber in the form of chitin, low fat, and B complex vitamins such as riboflavin, niacin, and biotin. In addition

to its good content, the texture of meat analog made from *F. venenatum* mycoprotein as the main raw material has a filament structure or long fibers that resemble muscle fibers in animal meat (Finnigan et al., 2019). The presence of mycoprotein produced during the fermentation process properly creates a fibrous structure that provides a chewy and meaty texture to the resulting meat analog product (Ritala et al., 2017). One of the meat analog products that is currently being industrialized using single cell fungal protein sources is Quorn. The product uses mycoprotein from *F. venenatum*. Quorn mycoprotein contains 45% protein, 25% fiber, 13% fat, and 10% carbohydrates in 100 g of dry weight (Finnigan et al., 2019).

Meat analog is made with the aim of being an alternative substitute for animal meat that has physical and chemical characteristics similar to animal meat. However, there are shortcomings attached to the process, such as the final result lacking the desired meat texture. The creation of a fibrous structure resembling animal meat can be done by using various protein sources, one of which is single cell mycoprotein. In addition, it can also be done with a bottom-up and top-down technology approach (Dekkers et al., 2018). The bottom-up technology approach involves the process of forming a structure from basic molecules that create protein fibers resembling muscle fibers in animal meat. The advantages of choosing bottom-up technology include being able to produce meat analog products with measurable and targeted protein content. However, choosing bottom-up technology in making meat analog requires sophisticated infrastructure and high costs because the technology used is complex (Ghorani & Tucker, 2015). The top-down technology approach is carried out with a protein that has been extracted from the main raw material, which is then processed mechanically and thermally to form a fibrous structure resembling the texture of animal meat. The advantages of choosing top-down technology include being more efficient and relatively inexpensive because it utilizes existing and simple technology. In addition, the selection of top-down technology is also more conventional. The food industry has widely used it to create meat analog, and it is suitable for various types of protein sources such as soybeans, peas, or other plant materials. However, with the selection of top-down technology in the manufacture of meat analog, the resulting fiber structure may be less precise than bottom-up methods, and there are limitations in modifying nutritional value because the physical process may reduce the nutritional content of the resulting meat analog (Smetana et al., 2018).

Animal meat contains myofibrillar protein as one of the main components, so it has a fibrous structure and texture. Myofibrillar protein plays a role in forming the basic structure of muscle fibers and provides a chewy texture when eaten, thus becoming a characteristic of animal meat. Making meat analog is one of the challenges related to creating a texture that resembles myofibrillar fibers; the use of vegetable protein in making meat analog that can be imitated of meat protein but is not identical to myofibrillar protein of animals. One of the vegetable proteins that has great potential to resemble the myofibrillar properties of meat is soy protein in the form of soy protein isolate and soy protein concentrate, which can be imitated through technology processes such as extrusion to obtain a fibrous structure resembling myofibrillar (Grabowska et al., 2016). In addition, wheat gluten protein or vital wheat gluten can also be used to form a chewy and fibrous texture resembling animal meat because of its nature, which includes a good water-binding capacity (Stanin et al., 2020). Processing technologies such as extrusion technology can create fibrous textures resembling muscle fibers in animal meat. High-pressure extrusion is a method for processing vegetable proteins such as soy and gluten, changing them into fibrous structures resembling myofibrillar ones. The extrusion process in the material causes the denaturation of protein molecules and makes the protein coagulate into a long structure resembling muscle fibers (Smetana et al., 2018).

Meat analog as a substitute for real meat faces challenges today because some consumers consider products made from vegetable protein to be less sensory-appealing (Chezan et al., 2022). This is because meat analog products made from plant-based ingredients do not produce the savoury and juicy taste and aroma typical of real meat. Efforts in creating meat analogs that have the taste and aroma typical of real meat involve adding additional ingredients such as spices. The presence of additional ingredients has been proven to significantly improve the sensory quality, especially the taste and aroma of the resulting meat analog.

Meat analog is created with the aim of resembling animal meat products in its fibrous structure and organoleptic properties. Meat analog, in its development, from the discovery and application of the right processing technology, needs to achieve optimal results in terms of texture, taste, and nutritional value. The discovery of meat analog processing technology including bottom-up and top-down technology, makes a significant contribution to the final quality of the meat analog produced. The scope of this study encompasses a review of several alternative protein sources including single cell mycoprotein, which has the potential to imitate animal meat muscle fibers naturally. The scope of this study includes understanding the nutritional value of mycoprotein, the technology used in the meat analog manufacturing process, and the challenges and future prospects in developing meat analog products that resemble animal meat products. This review aims to provide a clear picture of the latest scientific developments and technological options available to improve the quality of analog meat products.

2. Alternative Protein Sources

Protein sources containing protein, carbohydrates, fats, minerals, phosphorus, vitamins, and calcium can be found in animal meat, which play a vital role in health. However, there are various problems associated with consumption of animal meat as a source of protein, particularly excessive consumption. Other alternative protein sources can be found in plant protein, which can also play a good role in body health (Harnack et al., 2021). The increasing development of human eating patterns and increasing innovation in the food sector, especially consumer demand for healthy food products (free of cholesterol and low in saturated fatty acids), has given rise to the development of products that do not come from animal sources such as meat analog (Kumar et al., 2017). Meat analog is defined based on various research sources, namely food products that resemble animal meat in terms of taste, texture, appearance and nutrition (Kumar et al., 2017). Meat analog has advantages compared to animal meat. It can be formulated in such a way that it has better nutritional value than meat, is more homogeneous, cholesterol-free and low in saturated fatty acids (Chezan et al., 2022). Currently, the forms of meat analogs are dominated by processed meat types such as patties, ground beef, sausages and nuggets (Sha & Xiong, 2020). Based on the materials from which a meat analog is made, it can be categorized as plant-based (Yuliarti et al., 2021), insect-based (Smetana et al., 2018), fermentation-based (Hashempour-Baltork et al., 2020), or cultured-based (Moritz et al., 2015) (Table 1).

Table 1. Comparative analysis of alternative protein types in the manufacture of meat analog

Protein Category	Raw Material	Nutritional Value	Production Cost	Consumer Acceptance	Reference
Plant-based	Soybeans, nuts, and wheat	It has a protein content of around 35-50%, is a high source of fiber, and has anti-nutritional compounds.	Relatively low due to raw materials being easily available and large-scale production.	Consumer acceptance is good and has been widely accepted, especially products such as tempeh, tofu, and other plant-based meat products. However, it is constrained by the end products, such as taste and texture, which are less similar to animal meat.	(do Carmo et al., 2021)
Insect based	Crickets, grasshoppers and caterpillars	Has high protein content with rich healthy fats and micronutrients	Insect-based production is still on a small scale, and there is a lack of utilization.	Some cultures are well-accepted regarding insect-based main ingredients but are hampered by negative perceptions, tastes, and food images.	(Smetana et al., 2018)
Microorganism based	Mycoprotein (<i>F. venenatum</i>) and spirulina (algae)	Has a high protein content ranging from 40-60%, low fat and carbohydrates	Large-scale fermentation processes and high initial production costs	Relatively new, but well-received by some countries in the European market. In addition, the texture obtained also resembles animal meat fibers.	(Caporgno et al., 2020)

One of the vegetable sources can be found in soybeans, which contain about 30% crude protein. Soybean protein consists of a mixture of water-soluble and water-insoluble proteins. Processing of soybeans to produce new products has different protein content. Soybean isolate processed by alkaline or acid precipitation has a protein content of about 90%. Soybean juice extracted from soybean water and then dried into a powder product has a protein content of about 45-50%. Soybeans processed into flour have a protein content of about 50% (Hadi & Brightwell, 2021). Wheat gluten from wheat grains can also be used to manufacture meat analog products. Wheat gluten isolate has a protein content of about 75-80%. The use of wheat gluten in the manufacture of meat analogs can also be considered because of its significant functional characteristics in the extrusion process, which produces a fibrous structure resembling minced meat.

Insect-based proteins generally have a high protein content, polyunsaturated fatty acids, and various micronutrients (zinc, iron, copper, magnesium, and manganese) (Mintah et al., 2020). Research related to the use of insect-based methods in producing meat analog extrudates was carried out by testing a combination of insect flour (*Hermetia illucens*) and corn flour using the hot moisture extrusion method. The characteristics of the resulting extrudate were layers resembling analogous meat with fibrous, fragile, glassy and homogeneous characteristics (Alam et al., 2019). Other research was also carried out by processing *Alphitobius diaperinus* insect protein concentrate and soybean concentrate with twin screw high moisture extrusion to produce analogous meat that was fibrous and had a layered texture (Smetana et al., 2018). The use of insect-based meat in making meat analogue has potential, as seen in several studies that were carried out. However, there are still several obstacles in terms of appearance and the need for additional protein from other types. Apart from that, the use of insect species also needs to be considered regarding the potential for transmission of pathogens or viruses from these insects through post-harvest processing involving thermal processes such as boiling and frying (Baiano, 2020).

Single cell protein (SCP), produced from various microorganisms, can also be used as an alternative in making meat analogs. SCP can be produced from algae, fungi, and bacteria (Ritala et al., 2017). Microalgae can grow quickly and have high productivity per area when compared to soybeans or wheat. Microalgae protein can accumulate protein >50% in dry form (Caporgno et al., 2020). One meat analogous product based on a single cell fungal protein that is currently being industrialised is the Quorn brand. The product uses mycoprotein from *Fusarium venenatum*. Mycoprotein Quorn contains 45% protein, 25% fiber, 13% fat and 10% carbohydrates in 100 g of dry weight (Finnigan et al., 2019). Apart from *F. venenatum*, many other types of fungi are included in the SCP group. For example, *Rhizopus oligosporus* fungus is used to make tempeh and produces biomass-containing protein during the fermentation process. In contrast to *F. venenatum*, which can produce high protein biomass through large-scale fermentation under controlled conditions and the final product of this process is called mycoprotein, *R. oligosporus* technically belongs to the SCP group because it produces protein biomass during tempeh fermentation but does not produce a mycoprotein as the final product (Lübeck & Lübeck, 2022). SCP from mushroom species usually contains 30-50% protein, vitamin B complex, and other micronutrients (Finnigan et al., 2019). Several studies reviewing the potential of non-animal protein sources are shown in Table 2.

Table 2. Potential alternative protein sources for animal meat

Raw Materials	Type of Meat Analog	Protein Composition (%)	Parameter	Novelty	References
Faba beans (<i>Vicia faba</i>)	Meat analog extrudates	63,5%	Analyzing the feasibility of producing meat analogs from pork bean concentrate protein sources using high moisture extrusion techniques	The results show that meat analog production is feasible only when faba bean protein concentrate is processed using dry fractionation. They also show that the addition of 50% and 80% button mushrooms can improve the overall flavour.	(do Carmo et al., 2021)
Button mushroom (<i>Agaricus bisporus</i>)	Filler taco and carne asada (steak)	50% and 80%	Analyzing the sensory substitution of button mushrooms in taco and carne asada fillings	No significant novelty	(Miller et al., 2014)
Oyster mushroom (<i>Pleurotus sajor-caju</i>)	Analog meat extrudates	7,5%, 15%, 25%, and 35%	Observing the best extrusion conditions to produce analog meat made from a combination of oyster mushrooms and soy protein	The results show that the best extrusion conditions are obtained from a combination of extrusion temperature of 140°C, screw speed of 100-160 rpm, and the addition of 7.5% and 15% oyster mushrooms	(Mazlan et al., 2020)

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Table 2. Potential alternative protein sources for animal meat (continued)

Raw Materials	Type of Meat Analog	Protein Composition (%)	Parameter	Novelty	References
Hongkong caterpillar (<i>Tenebrio molitor</i>) and cage caterpillar (<i>Alphitobius diaperinus</i>)	Minced meat	83,8%	Analyzing the microbial count in the production and storage process of minced meat made from mealworms and caterpillars	The results show that mealworms and caterpillars can be used to produce minced meat with a low microbial count	(Stoops et al., 2017)
Cage caterpillar (<i>Alphitobius diaperinus</i>)	Meat analog extrudates	15-50%	Analyzing and making meat analogs made from a mixture of caterpillar protein concentrate and soybeans using the high moisture extrusion technique	The results show that the combination of soy protein concentrate and mealworms (15-50%) can produce a product with hardness and composition that resembles conventional meat	(Smetana et al., 2018)
Quinoa (<i>Chenopodium quinoa</i> Wild) dan Buckwheat (<i>Fagopyrum esculentum</i> Moench)	Burger	15% and 30%	Analyzing the physicochemical, nutritional and sensory characteristics of beef burgers containing quinoa and buckwheat flour	The results showed that the quinoa and buckwheat in the resulting burgers had minerals such as magnesium, phosphorus, iron, and zinc, which were higher than those in the soy protein burgers	(Smetana et al., 2018)
Mycroalga <i>Auxenochlorella protothecoides</i>	Meat analog extrudates	5-50%	Analyzing the best formulation for making analog meat extrudates with the addition of microalgae	The results show that a combination of 30% microalgae is the best extrudate, and the moisture content is 60%	(Caporgno et al., 2020)

3. Nutritional and Health Value of Single Cell Mycoprotein for Meat Analog

The nutritional content of products, especially meat analog products that are to be marketed to the public, is certainly an important factor in a food product. The resulting meat analog product is expected to have a high protein content with a complete essential amino acid profile and be easily digested. Some nutritional comparisons of various types of meat analogs are shown in Table 3.

Research that had been conducted on the nutritional value of various commercial meat analog products, were compared to cooked ground beef. Meat analogs from various well-known brands such as Beyond Burger®, Impossible™, and MorningStar Farm® have protein, total fat and iron contents that tend to be close to cooked ground beef products. However, the results obtained showed a clear difference in carbohydrate content that was not found in cooked ground beef. In addition, the salt content in meat analog also tended to be higher when compared to cooked ground beef (Bohrer 2019).

Other researchers showed that plant-based meat (PBM) from soybeans had the highest average protein, omega-3 fatty acid and fiber content when compared to PBM from other ingredients (wheat, a mixture of wheat with soybeans, and nuts). The product being compared to the soybean-based one, which was a source of nut protein, had the advantage of containing monounsaturated fatty acids (MUFAs). Apart from that, there are also PBMs with the addition of eggs, which show an improvement in sensory characteristics and have a significantly increase amount of polyunsaturated fatty acids (PUFAs) (Fresán et al., 2019). The nutritional advantages of PBM include low saturated fat, zero trans-fat and cholesterol (Bohrer, 2019). Apart from that, PBM also contains carbohydrates such as fiber, starch and oligosaccharides.

Research on insect nutrition was carried out using honey bees and crickets, showing that the average iron content was around 18.5 mg and 5.46 mg, which was higher compared to beef (1.95 mg). Apart from that, the research studies also found the higher calcium and riboflavin contents in honeybees, crickets, silkworms, and caterpillars than those of livestock such as cows and chickens. However, it is important to consider insects as a substitute for real meat that some types of insects have high saturated fat and sodium content. One example is adult crickets, which have a sodium content of around 152 mg/100 g. In comparison, beef has a sodium content of around 9.84 g/100 g, so cricket-based meat analog is not a suitable substitute for real meat when a heart disease prevention is concerned (Payne et al., 2016).

One protein source for making meat analog, which has been commercialized in 17 countries, including the United States, is the Quorn product from mycoprotein produced from the fungi *F. Venenatum*. It has a good nutritional profile, making it an alternative protein source. The mycoprotein from *F. venenatum* has a high protein content value ranging from 11-15 g/100 g and fiber ranging from 6-8 g/100g, total fat ranging from 2-3 g/100g, carbohydrates ranging from 2-4 g/100 g, minerals in the form of iron, zinc, magnesium, and phosphorus, cholesterol, sodium, and sugar (Saeed et al., 2023). Based on dry weight, the mycoprotein has a protein content of 45% and fiber of 25%. Mycoprotein is also known to be rich in essential amino acids, with a total protein percentage composition of 41% (van Vliet et al., 2015). Mycoprotein also contains less than 1.5 g of long and short-chain saturated fatty acids per 100 g of solids. It contains several water-soluble vitamins, such as pyridoxine (0.1 mg), folate (114 mg) and cobalamin (0.72 mg) (Saeed et al., 2023). The high protein value of mycoprotein provides nutritional benefits for

Table 3. Comparison of nutrition of various types of meat analog and beef at 100 g

Product	Energy (kcal)	Protein (gr)	Total Fat (gr)	MUFAs (gr)	PUFAs (gr)	Carbohydrate (gr)	Fiber (gr)	Na (mg)	Fe (mg)	References
PBM wheat	176,52	21,68	5,68	1,44	3,05	10,95	1,35	251,20	2,38	(Bahmanyar et al., 2021)
PBM soy	234,62	24,96	6,63	1,64	3,63	20,31	6,35	267,06	6,05	
PBM wheat/soy	185,52	21,44	5,64	1,47	3,01	13,94	2,71	189,65	3,06	
PBM nuts	204,60	18,12	11,59	5,13	4,11	9,63	3,01	162,18	3,62	
PBM with telur	202,11	19,87	8,15	2,17	4,38	13,91	2,57	206,99	3,23	
Beyond burger®	221,24	17,70	15,93	-	-	2,65	1,77	345,13	3,72	(Fresán et al., 2019)
Impossible™	212,39	16,81	12,39	-	-	7,96	2,65	327,43	3,72	
MorningStar Farm®	203,13	25,00	7,81	-	-	12,50	6,25	609,38	1,72	
Raw ground beef	152,00	20,85	7,00	-	-	0,00	0,00	66,00	2,33	
Cooked ground beef	182,00	25,56	8,01	-	-	0,00	0,00	72,00	2,82	
McDonald's beef patty	266,67	23,33	20,00	-	-	0,00	0,00	400,00	3,33	

the body, including controlling blood sugar, cholesterol, glucose, and insulin levels (Ahmad et al., 2022).

A survey conducted on several products such as burgers, sausages, ground beef, chicken, seafood and other types of meat analog in the Australian market showed that the average meat analog product obtained had a salt composition below 500 mg/100 g, other meat analog products were also found to have a salt composition of up to 1200 mg/100 g (Curtain & Grafenauer, 2019). Consuming too much salt is known to have a negative effect on health, so according to the UK Public Health Agency, the salt content in meat alternatives for target consumers should be reduced to 250 mg for plant-based meat alternatives and 360 mg for other meat alternatives. In addition, excessive salt consumption can also increase blood pressure and cause various other cardiovascular diseases (Hendriksen et al., 2014).

4. Meat Analog Processing Technology

The technology for processing meat analog to produce real meat texture depends on the type of meat product desired to imitate. Meat products to be imitated are categorized into ground meat, minced meat and whole fiber meat products. The aim of making meat analogs is to obtain whole-fiber meat products with organoleptic properties similar to real meat. Technology in commercial meat analog processing is classified based on its fundamentals with the aim of creating a complete fibrous structure resembling real meat, namely bottom-up technology and top-down technology (Dekkers et al., 2018). Several meat analog processing technologies are shown in Table 4.

4.1 Bottom-up technology

Bottom-up technology creates analogous fibrous meat structures that resemble the muscle structure of animal meat. Components that have been formed using bottom-up technology are then assembled or combined with several other materials, creating a larger final product. Several studies have been carried out regarding meat analog processing using bottom-up technology (Table 5).

4.1.1 Spinning technique

The spinning technique works by releasing a solution containing protein through a spinneret, then immersing it in a bath containing a non-solvent for the protein. The immersion process could cause the exchange of solvent and non-solvent, resulting in the deposition and solidification of the extruded protein phase, and forming stretched filaments with a thickness of around 20 μm (Dekkers et al., 2018). The type of structure formed may depend on the solidification mechanism that occurs, e.g., the desired fiber structure is obtained when the dispersed phase solidifies. The capillary-filled gel structure is obtained when the continuous phase is solidified. The dispersed phase remains liquid, and the fiber-filled gel structure is obtained when the dispersed phase and the continuous phase are compacted (Nieuwland et al., 2014). The use of spinning techniques has an impact on the environment, such as the large amount of chemical waste production due to the use of solvents. This can be minimized by using solvents that can be recycled. Processing with spinning techniques has several advantages. The techniques can be done on a large scale, creating a fiber texture that is similar to animal meat fibers. Moreover, in the process,

Table 4. Meat analog processing technology to create a fibrous structure

Technology	Technique	Protein sources	Parameter Process	References
Bottom-up	Spinning	Vegetable	The process of making meat analog is complex. This is because it requires a high concentration of vegetable protein solution and has high costs in large-scale applications.	(Dekkers et al., 2018)
	Electrospinning	Animal	The process of making meat analog is complex. This is because it requires proteins that must be very soluble and not globular.	(Ghorani & Tucker, 2015)
Top-down	Freezing	Vegetable	The process of making meat analog is complex. This is because it requires proteins that have good solubility.	(Chantanuson et al., 2022)
	Sliding cell	Animal	The process of making meat analog is simple. This is because it requires ingredients from several mixtures.	(Krintiras et al., 2016)
	Extrusion	Vegetable	The process of making meat analog is commonly used. This is because the resulting product resembles real meat.	(Smetana et al., 2018)

Table 5. Meat analog processing using bottom-up technology

Technique	Raw Materials	Equipment Type	Novelty Research	References
Spinning	Soybean protein isolate	Isolate protein	The selected concentration of soy protein isolate in making meat analogs affects the texture resembling real meat with strength and elasticity that can be controlled through process parameters.	(Joshi et al., 2023)
	Wheat protein	Coagulation	The raw materials in making meat analog can provide the resulting protein fiber, which has a high nutritional value such as high protein and essential amino acids.	(Nowacka et al., 2023)
Electrospinning	Sodium caseinate, protein isolate, zein	Electrospinning setup (cable, high voltage, ground electrode)	The results show that the meat analog obtained has a fiber structure resembling animal meat on a nanometer scale.	(Nieuwland et al., 2014)
	Gelatin and zein	Immersion rotary jet spinning	The results show that the production of fibrous gelatin supports the making of microfibrinous fibers.	(Gagaoua et al., 2022)

protein or polymers are used in making meat fibers efficiently. However, spinning techniques require a high concentration of vegetable protein solution, and the quality of the fiber produced is still not optimal when compared to electrospinning. Furthermore, the technique is of relatively high cost when performed in large-scale applications (Dekkers et al., 2018).

4.1.2 Electrospinning technique

The electrospinning technique works by pushing a biopolymer solution through a hollow needle or spinneret, which has an electrical potential relative to the ground electrode. Charge accumulation on the surface of the droplet emerging from the spinneret will cause surface instability so that the product forms thin fibers that are attracted to the ground electrode (Ghorani & Tucker, 2015). Electrospinning techniques are generally utilized in nanofiber applications, which are used as carriers or delivery systems for bioactive components in the form of polyphenols and probiotics (Librán et al., 2017). Still, they can also be applied to produce fiber in meat analogs (Nieuwland et al., 2014). The use of electrospinning techniques can have an impact on the environment, such as producing energy waste due to the use of strong electric fields. This effect can be minimized by the use of more renewable energy sources to reduce the impact of high energy consumption. Processing with electrospinning techniques is mostly reported on several animal proteins such as whey, collagen, and eggs, but only a few applications on vegetable proteins. This is because the electrospinning technique can only be used on proteins that are highly soluble, have random coils, and are not globulins. These requirements are generally not met by vegetable proteins because vegetable proteins have a spherical shape and, after denaturation, form insoluble aggregates. Processing with electrospinning techniques offers a number of advantages, such as a manufacturing process that produces finer and more structured fibers resembling muscle fibers in animal meat. However, the electrospinning process is more complex and requires special equipment and settings. This makes electrospinning techniques being less commonly used by large industries due to limited scalability (Ghorani & Tucker, 2015).

4.2 Top-down technology

Top-down technology creates whole-fiber meat analog products by mixing several raw ingredients in a formula to be processed into raw dough. The raw dough obtained is then molded on a machine so that it gets a fibrous texture, which is formed due to the combination and functional properties of the materials used. Several studies have been carried out regarding meat analog processing using top-down technology (Table 6).

4.2.1 Freezing technique

The freezing technique works by first freezing the protein solution to produce a structure. After that, it is heated in the same direction as the protein solution so that ice crystal needles are formed and a porous microstructure is produced. The size of the ice crystal needles can be adjusted according to the temperature and freezing rate (Chantanuson et al., 2022). Next, the frozen product obtained is then dried using a freeze-drying technique without melting the ice crystals with the aim of obtaining a porous microstructure with the proteins arranged like parallel sheets (Dekkers et al., 2018). The sheets obtained are

Table 6. Meat analog processing using top-down technology

Technique	Raw Materials	Equipment Type	Novelty Research	References
Freezing	Soybean flour (SBF), Soy protein isolate 1 (SPI1), and Soy protein isolate 2 (SPI2)	Rheometer with triangular probe for measurement of sample breaking strength	A freezing approach to creating a fiber structure in a protein emulsion with a fiber structure formed from meat analogs produces a porous microstructure resembling animal meat muscle.	(Chantanuson et al., 2022)
	Wheat Protein	Coagulation	The raw materials in making meat analog can provide the resulting protein fiber, which has a high nutritional value such as high protein and essential amino acids.	(Nowacka et al., 2023)
Sliding cell	Sodium caseinate, protein isolate, zein	Electrospinning setup (cable, high voltage, ground electrode)	The results show that the meat analog obtained has a fiber structure resembling animal meat on a nanometer scale	(Nieuwland et al., 2014)
	Gelatin and zein	Immersion rotary jet spinning	The results show that the production of fibrous gelatin supports the making of microfibrinous fibers.	(Gagaoua et al., 2022)
Extrusion	Soybean flour, protein isolate	The extruder uses twin screws.	The results show that the meat analog obtained is layered and fibrous, resembling animal meat, which can be seen on a microscopic scale.	(Zhang et al., 2023)
	Soybean protein and mealworms (<i>Alphitobius diaperinus</i>)	The extruder uses a high-moisture extrusion technique.	The results show that the combination of soy protein concentrate and caterpillar can produce a product with hardness and composition that resembles conventional meat.	(Smetana et al., 2018)

connected to form a cohesive fibrous product. The use of freezing techniques requires that the protein has good solubility before being frozen. During the freezing process, the protein becomes insoluble in order to obtain a fibrous product. The use of freezing techniques has an impact on the environment in that it requires a lot of energy and thus results in high carbon emissions. This is especially so for long-term storage, so the sustainability of the freezing techniques needs to be reviewed. However, one advantage of processing with freezing techniques is that it is a fairly simple manufacturing process that can be applied on a small to large industrial scale with a fibrous texture that resembles animal meat because it goes through the process of forming ice crystals. However, with the freezing technique, the resulting product reduces nutritional value because freezing can cause the degradation of several nutrients, such as vitamins that are susceptible to low temperatures. Moreover, the consistency of formation of the resulting fibers is difficult to control (Yuliarti et al., 2021).

4.2.2 Sliding cell technique

The shear cell technique involves the application of shear forces to proteins, which works by producing fibrous products from a mixture of calcium caseinate with several vegetable protein mixtures, for example, soy protein concentrate, soy protein isolate – wheat gluten, and soy protein isolate – pectin (Grabowska et al., 2016). The final structure of the shear cell technique depends on the materials and processing conditions used. The shear cell technique using calcium caseinate produces structures that show anisotropy at the nanoscale. In contrast, vegetable proteins produce structures that show observable anisotropy up to the micrometre scale. The use of shear cell techniques on the impact on the environment is minimal because the use of mechanical force in the process to change the protein structure requires only a little energy. Processing with shear cell techniques has advantages, such as producing meat analog products with a uniform and smooth texture due to the precision in creating a regular texture. However, with the shear cell technique, special equipment is required, production costs are high, and the process is complex compared to other techniques (Krintiras et al., 2016).

4.2.3 Extrusion technique

The extrusion technique is a common commercial technique used to convert vegetable materials into fibrous products resembling real meat. The process involves mixing raw protein materials, which are then extruded through a nozzle at high temperature and pressure. This technique is widely applied in the manufacture of meat analogs. Extrusion techniques are divided into two classes of process arrangements, namely low moisture and high moisture. Using the low moisture extrusion technique, flour or concentrate is mechanically processed into textured vegetable protein (TVP) (Emin & Schuchmann, 2016). Products resulting from the low moisture extrusion technique are dry products or have low water content (water content <30%) and also have limited acceptance due to the taste in the mouth that is less suitable for consumers. Meanwhile, for the use of high moisture extrusion techniques, the protein from the materials used is melted in a barrel with a combination of heating, hydration, and mechanical deformation. Protein from the material in the barrel, when melted, flows into a mold that is parallel to the laminar flow and cooled to prevent expansion so that the resulting product is fibrous with high water content (water content > 50%) and has a fresh quality with a texture resembling real meat that consumers can accept. The use of extrusion techniques also has an impact on the environment, such as the use of large amounts of energy, because it involves high

temperatures and pressures during the processing process. Therefore, sustainability should be sought through innovation in the use of renewable energy and improvement of extrusion efficiency. Processing with extrusion techniques has advantages such as the acceptance of texture quality that resembles animal meat well and operational efficiency that can be applied to large-scale production for industry. However, extrusion techniques require temperature and pressure; so careful control of the process is needed to minimize the energy released. There are also limitations on the types of products that can be created through the extrusion process due to the high temperatures and pressures (Smetana et al., 2018).

5. Potential and Challenges of Developing Meat Analog

Various aspects for the development of food products that are widely distributed to consumers certainly needs to be considered so that these products can fulfil consumer desires or can even improve nutrition and thus consumer health. The production of livestock meat has various negative impacts, one of which is quite large, namely the effect on environment (Gerbens-Leenes et al., 2013). Meat analog is not only an alternative but also a more environmentally friendly food option for consumers, especially in terms of water use, land use, and greenhouse gas emissions in comparison to animal meat production (Angelis et al., 2024). For the evaluation of the environmental impact of animal meat production based on the research work by Nisov et al. (2022), water is required in a large amount of water, with an average of 15,000 L of water needed to produce 1 kg of beef, while meat analog made primarily from plants such as soybeans only requires less water, around 300-400 L of water per kg of product. As for land use, animal meat requires a large area for its production, around 20-30 m² of land and even additional land is needed to grow animal feed, while meat analog made primarily from plants only requires a small amount of land, around 1-2 m² of land per kilogram of product. Greenhouse gas emissions also have an impact on the environment. Animal meat production contributes around 60 kg CO₂ eq/kg due to enteric fermentation and manure processing, while meat analog made mainly from plants only produces around 3-4 kg CO₂ eq/kg (Desiderio *et al.* 2023). Research has been carried out on the life cycle of analog meat, with results showing that meat analog can be a sustainable alternative (Smetana et al., 2015). This can be seen from the meat analog processing process, which is less complicated compared to the general meat processing chain. The meat processing chain generally extends from the harvesting of animal feed to entry into the slaughterhouse. Meat analog, which has a shorter production chain, results in a lower carbon footprint (Fresán et al., 2019).

The demand of consumers regarding healthy food products (free of cholesterol and low in saturated fatty acids) has increased markedly in the last decade. Consumers implement plant-based diets with the aim of reducing the consumption of animal food products such as meat, eggs, milk, and dairy products (Fehér et al., 2020). These changes are a great opportunity for the development of analog meat products. The existence of meat analog products also has advantages compared to animal meat because they can be formulated in such a way that they offer better nutritional value than animal meat. They can contain complete protein and dietary fiber, are low in saturated fatty acids, and do not contain cholesterol (Bohrer, 2019).

A quite controversial problem also exists regarding the antibiotic content in animal meat in the livestock sector. Antibiotics are usually used to treat disease, but can also improve the development of farm animals. The effects of these antibiotics can have negative consequences for humans who consume them, such as bacterial resistance and

toxic effects from residues in food (Wang et al., 2017). Meat analog, however, is artificial meat that is free from antibiotics because all the constituent ingredients come from plants (Yuliarti et al., 2021), insects (Smetana et al., 2018), microalgae (Caporgno et al., 2020), and mycoprotein (van Vliet et al., 2015).

Another problem found was that slaughtered meat is generally closely associated with the occurrence of foodborne illness, which can be caused by pathogenic microbes that contaminate the meat product. Factors causing contamination can vary and include the control of the environment of livestock and the handling of the meat. These factors can cause contamination to occur, which can then cause various health problems for consumers. This problem is certainly not found in meat analog products; this is because there are no risky environmental factors, so product safety is more guaranteed (Heredia & García, 2018). Apart from that, safety aspects in meat analog products can be considered through processing, such as preservation, so that they can have a long shelf life. Modern preservation methods that are suitable for meat analog products in general are preservation at low or non-thermal temperatures (Dekkers et al., 2018).

Product development certainly cannot be separated from the challenges that accompany it, and of course, these challenges must be anticipated well so that consumers can accept the resulting product. The development of meat analog products has several challenges, including sensory characteristics that may only partially represent real meat, such as less perceived meat flavour (Graça et al., 2016). A beany aroma comes from bean protein isolate, and the aftertaste is not suitable. Apart from that, appropriate technology to process meat analog in such a way that its structure is resemble real meat is still being research (Sha & Xiong, 2020).

The majority of meat analog developments use soy and wheat proteins as protein sources (Bohrer, 2019). However, soy and wheat ingredients are known to be Category 8 allergens, which cause 90% of allergic reactions for consumers with allergies (Mark & Venter, 2020). The presence of soy protein and gluten is a serious consideration when developing meat analog products that are safe for all consumer categories. The success of meat analog product development is related to consumer decisions to buy the product, one of which is price (Elzerman et al., 2011). The raw materials for making plant-based meat analog are of low cost when compared to microorganism-based proteins. However, single-cell-based proteins such as mycoprotein provide advantages such as having a texture quality that resembles animal meat, even at a high cost. An increase in production scale in the future will allow for a decrease in prices. Wider use of technology is expected for the price of meat analogs competitive with animal meat, making it a sustainable choice. Other efforts that need to be made include product marketing techniques, such as clear labels and consistent and clear promotions to consumers (de Boer et al., 2014). In the future development of meat analogs, producers are expected to be able to overcome difficulties regarding the texture, taste, and aroma of the meat analog products produced. In addition to the sensory aspects that need to be studied, nutritional value and consumer safety, cost optimization and environmental impact need to be considered (Hwang et al., 2020).

6. Conclusions

Meat analog is considered as an alternative to animal meat due to a number of perspectives. Alternative proteins in the manufacture of meat analog can be selected from sources that do not cause environmental impacts or other impacts. Protein sources for the development of meat analog products based on research results show that they can be

categorized into several large groups, namely plant-based, insect-based, fermentation-based, and culture-based. In addition to having good nutritional value, the resulting meat analog products can also have a positive impact on health as they can be cholesterol-free and low in saturated fatty acids. One of the main findings in this study is that single cell mycoprotein, especially from *F. venenatum*, is a potential protein source in the manufacture of meat analog. The mycoprotein obtained has a fibrous texture resembling animal meat and has nutritional values such as being rich in essential proteins, low in fat, and high in fiber, making it a healthy alternative to animal meat. Mycoprotein production is a sustainable solution in terms of impact on the environment in terms of water, land, and greenhouse gas emissions, which are much lower than animal meat production. Meat analog processing technology can be done with bottom-up technology and top-down technology. Bottom-up technology such as spinning techniques require a high concentration of vegetable protein solution that are of relatively high costs in their application. Moreover, electrospinning techniques require proteins that are of high solubility and are not globular proteins. Top-down technologies such as freezing techniques require proteins that must be of good solubility before being frozen. Examples also include sliding cell techniques that require a mixture of calcium caseinate and several types of vegetable protein, and extrusion techniques that are often applied in the industry because they affect the structure of the material produced both chemically and physically due to high-temperature pressure and shear. The meat analog industry that utilizes mycoproteins has the potential to meet consumer demand because consumers want products with a texture that resembles animal meat, with health benefits, and environmentally friendly. Therefore, the challenge that must be solved by producers and researchers in the development of meat analog products in the future is to find technology and additional materials that can be added to obtain good composition and texture in terms of sensory attributes. With the development of new processing technologies, exploration of new protein sources, and environmental sustainability, the meat analog industry has great potential to overcome future challenges and meet consumer demand for healthy, environmentally friendly products that resemble animal meat in terms of taste, texture, and nutritional value.


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8. Conflicts of Interest

The authors confirm that this work is original and has not been published elsewhere, nor is it currently being considered for publication elsewhere. The authors declare that they have no conflict of interest.

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