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Meat Analogues: Types, Methods of Production and Their Effect on Attributes of Developed Meat Analogues

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Abstract

Population explosion and low purchasing power of people in developing nations are a reason for low affordability of sources of high biological value proteins like animal meat. Also, producing healthy and delicious meat-free food in order to satisfy vegetarians and promote personal health is becoming common. Vegetable proteins such as textured soy protein, mushroom, wheat gluten, and pulses are increasingly being used to replace animal protein sources. This paper attempts to describe the various types of meat analogues that are commercially produced and are available in the market nowadays. The different meat analogue products and their protein sources have been discussed in detail along with a few novel ones like single-cell proteins from bacteria. Recent studies regarding the different approaches for production of meat analogues have been discussed with examples. 3D printing of the meat analogues has also been covered in the review keeping in mind its wide applicability for producing a range of meat analogues. Another distinctive area of meat analogue production focused on this review is the effect of various techniques on the different attributes of meat analogues. The effects of production methods on the attributes of meat analogues like texture, colour, flavour, cutting force, cooking yield, water, and oil absorption index have been discussed keeping in mind the diversity of the nature of the meat analogues.

Keywords Meat analogue · Biological value · Vegetable protein · Meat-free · Bottom-up strategy · Top-down strategy

Abbreviations

3D	Three dimensional
AI	Anisotropy index
DI	De-ionized
DS	Dietary supplement
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
EHEC	Ethyl hydroxy-ethyl cellulose
GDL	Glucono-delta-lactone
GHGE	Green-house gas emission
GRAS	Generally recognized as safe

HDL	High density lipoprotein
HME	High moisture extrusion
HMMA	High moisture meat analogue
HPC	Hemp protein concentrate
IVPD	In vitro protein digestibility
LCA	Life cycle analysis
LDL	Low density lipoprotein
MC	Methyl-cellulose
OSA	Octenyl succinic anhydride
PB	Phosphate buffer
PD	Protein digestibility
PPN	Pea protein nugget
RNA	Ribo-nucleic acid
RPM	Rotations per minute
SCP	Single cell protein
SDS	Sodium dodecyl sulphate
SEM	Scanning electron microscope
SPI	Soy protein isolate
TPP	Texturized peanut protein
TSP	Textured soy protein
TVP	Textured vegetable protein
WAI	Water absorption index
WG	Wheat gluten

Novelty Impact Statement

A growing body of evidence implies that meat consumption has detrimental effects on health and the environment, which makes it imperative to encourage shifting away from animal-based diets to more plant-based ones. By providing vegans, vegetarians, and meat-lovers with meat alternatives and meat analogues, this can be achieved while satisfying their dietary needs and sensory appeal.

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Introduction

The main component of most of the diets across the globe is meat, and it is considered a staple component of many cuisines. Meat contains significant amounts of protein and is highly nutritious. Meat is a nutritious food with a high nutritional value due to abundance of macro- and micro-nutrients, including iron, zinc, vitamins B1, B12, A, D, niacin, and proteins (Laskowski et al., 2018). The demand for meat is growing due to several factors. For enough proteinaceous food to be produced for everyone as well as to control other harmful effects of meat production, we need to switch to plant-based foods for protein needs.

As a result of livestock rearing, the environment is often affected by harmful emissions, excessive energy consumption, excessive soil and water consumption, and poor utilization of other resources (Nijdam et al., 2012). In addition, meat consumption poses several health-related risks due to the presence of pathogenic microbes such as *Salmonella enterica* and *Escherichia coli*, which can cause severe food poisoning if not handled correctly. Additionally, further health complications (such as colon cancer and breast cancer in women) related to consumption of red meat and hormone usage in meat production (for faster and higher meat production) are also a thing to concern about (McAfee et al., 2010). Furthermore, aspects of animal welfare that concern social and cultural settings are also considered by some individuals for lowering meat production and consumption. The various issues related to the production of animal meat are given in Fig. 1.

Although there are numerous arguments in favour of a plant-based diet, there are very few people adopting such a

diet around the world. And there is a very good reason why most people adhere to animal meat diets. They perceive meat as a highly nutritious and delicious food commodity. The most effective way to reduce meat consumption is through consumer awareness education. In addition to reducing meat in the diet, it is also important to increase the consumption of plant-based foods high in protein for example beans, legumes, nuts, and other similar foods (de-Boer et al., 2014). Another method would be to completely replace meat with a plant-based source. Plant-based components can be employed for creating these food products that are as nutritious as meat and also mimic the meat in terms of taste and appearance. Tofu and tempeh, which are traditional foods of Asian markets, fall into this category because they are prepared in a similar manner, and they are quite popular in Asian countries contrary to Western countries (Asgar et al., 2010). Therefore, nowadays meat analogues are being prepared as they are perceived healthier and better suited to Western diets.

Analogues of meat are products that have certain properties (like taste and texture) identical to animal meat and are manufactured to mimic animal products. A meat analogue is a compound which, despite its structural similarity, differs in composition from its counterpart. Meat analogues share much of the same structure as meat but differ slightly in composition. They have also been called mock, imitation, and faux meat techniques. In order for meat substitutes to fulfil the chemical attributes of meat (mainly flavour, texture, and appearance), the substitutes need to be made from meat-based compounds, such as surimi, which is a cheap and healthier alternative to meat.

Fig. 1 Issues related to the production of animal meat.
Source: Post (2012)

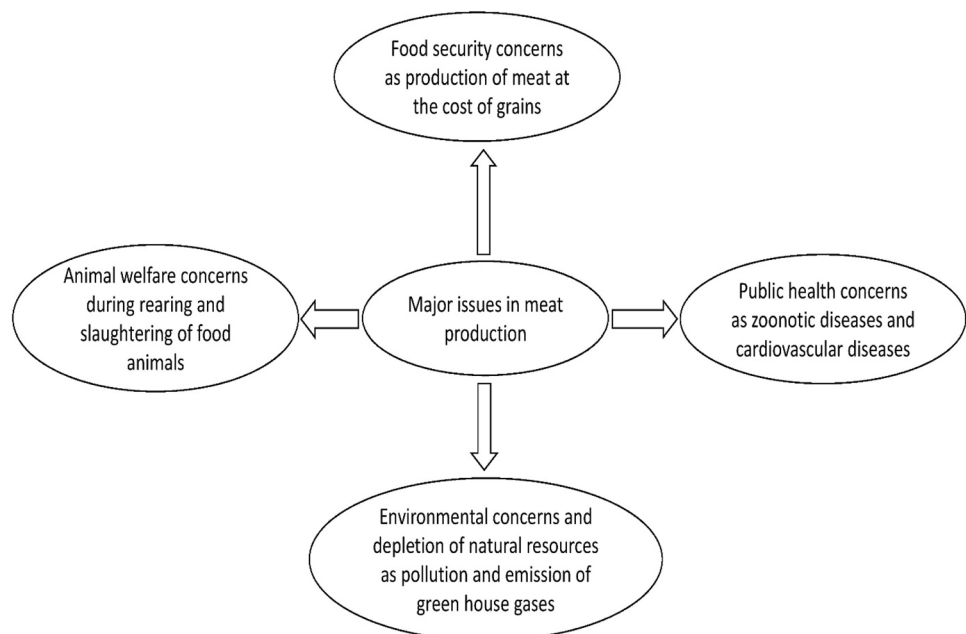


Table 1 Plant-based meat analogue ingredients and their main role

Ingredients	Sources	Main role
Non animal proteins	-Plant sources: soy, wheat, legumes, pea, lupin, rice, and potato -Novel sources: microalgae and seaweed	Nutrition, structure, colour, texture, and flavour tech-functional properties
Lipids	-Rich in saturated fatty acids (e.g., coconut oil and cocoa butter) -Rich in unsaturated fatty acids (e.g., sunflower oil, canola oil, sesame oil, and avocado oil) -Fat replacers: oleogels and fibres	Flavour, texture, and mouthfeel
Polysaccharides	-Native starches -Flours -Fibres	Consistency and water binding
Flavouring ingredients	Savoury yeast extract, paprika, sugar, spices, and herbs	Flavour
Colouring agents	Lycopene, beet juice extract or leghaemoglobin	Meat colour
Fortification ingredients	Tocopherols, zinc gluconate, thiamine hydrochloride, sodium ascorbate	Nutritional value

Source: Boukid (2020)

The different examples of the ingredients used for developing meat analogues with their main role have been given in Table 1. A meat analogue will supply its consumers with all the biological, nutritional, and characteristic qualities of real meat while containing nutritional and characteristic elements that are not found in meat. Most of the market of meat analogues consists either of the vegans, vegetarians, or the meat-eaters looking for ways to cut back on meat consumption for socio-cultural reasons or for their well-being (e.g., people with religious concerns). Developing products that can function proactively to minimize the consumption of animal meat is considered a promising way to decrease the number of people consuming animal meat (Elzerman et al., 2011). As a result, the unique characteristics of meat, such as taste, colour, texture, flavour, and sensory properties need to be duplicated in the plant-based meat substitutes since these are the benchmark attributes of animal meat that most of the meat-eating consumers relish (Grunert et al., 2004). The aim of the paper is therefore to review the various types of meat analogues, their production process, and how the process itself and the processing conditions affect the quality and sensory attributes of the developed meat analogues.

Meat Analogues (Plant Based and Animal Based)

Fruits, vegetables, and fibres generally make up most of a vegetarian diet as their health benefits have been well documented. Many protein sources can be utilized as substitutes for meat. Vegetable proteins like gluten, cottonseed protein, sesame protein, peanut protein, yeast, and soybean globulins are presently the most common raw material source for meat analogues. Currently, the bulk of the production comes from soybeans, which is still the most economically viable raw material for production of meat analogues (Kumar et al., 2017). Meat analogues could be made with proteins of high biological value or to contain such proteins in high quantities and could be added to a monotonous diet using only plant protein. The different protein sources and the technology used for manufacturing of meat analogues have been given in Table 2. For meat analogue production, soybean and several oilseed extracts, including proteins derived from fermentation involving a variety of substrates and microbes, would be mainly employed. New sources of protein, such as fungi and bacteria, are becoming available and offer unique technical and functional properties, flavour, and nutritional values, depending on their origin

Table 2 Meat analogue sources and the technology behind their manufacturing

Product	Source	Technology used	References
Cultured meat	Skeletal muscle cells	Tissue growth in a bioreactor	Stephens et al. (2018)
Microalgae	<i>Chlorella</i> and <i>Spirulina</i> species	High moisture extrusion	Palanisamy et al. (2019)
Tofu	Soybean milk or curd	Salt-induced or acid-induced coagulation of soymilk	Kumar et al. (2020)
Seitan	Wheat gluten	High moisture extrusion	Joshi and Kumar (2015)
Textured soy protein (TSP)/soy meat	Defatted soy flour	Extrusion	Kyriakopoulou et al. (2019)

and processing method. Creating improved and new meat analogue properties and taste sensations have been made easy through the use of new proteins and their combinations (Wild et al., 2014). In addition to hydrating and preparing a texturized vegetable protein (to be used for meat analogue production), a protein base made from milk protein, wheat gluten, and water can also be used for producing meat analogues (Howse et al., 2005). Konjac fibre-based products, containing non-digestible dietary fibres, are the other mock-meat products available to consumers as a choice when it comes to taste and health. The industrial processes produce meat analogue items using various protein-rich precursor substances. These substances are gluten from cereals (rice, wheat, or maize; vital or residual starch), defatted oilseeds, flours from cereals or beans, meals, and derivatives (e.g., defatted soya flour, wheat flour, soya protein concentrates), or meat-based precursors (e.g., by-products of mechanical disintegration of meat, fish meal, and dry egg white) (Kumar et al., 2017).

Mushroom

Mushrooms are generally fibrous in nature, and the inclusion of mushroom in the product enhances chewability. Their cell membranes are abundant in polyunsaturated fatty acids, while the hyphae wall is abundant in dietary fibres. They also contain high levels of protein in their cytoplasm. As a result of mycoprotein intervention, total cholesterol and low-density lipoprotein (LDL) cholesterol are reduced, and high-density lipoprotein (HDL) cholesterol is increased (Coelho et al., 2020). One can get all of the protein, minerals, and vitamins they need from the consumption of mushrooms. As an alternative of meat, *Fusarium graminearu* was the first edible filamentous fungus to be used in commercial products like burger patties and sausages. The sugars and amino acids in fungi enhance the palatability of food and impart sweetness and umami taste to resemble meat (Oosone et al., 2020). Products derived from mushrooms are generally more delicious than other plant-based or plant-derived products. To make mycoprotein-based meat substitutes, fermented mushrooms are mixed with eggs, seasonings, and flavourings. It is commonly substituted for beef and chicken in European countries.

Researchers and food technologists have been forced to search for better alternatives due to *Fusarium* is thought to be pathogenic rather than a true mould. The benefits of fungal protein over bacterial and vegetable proteins are as follows:

1. Compared to the beany flavour of textured soy protein, the mushroom flavour of fungal protein is more appealing.
2. The fungal proteins produced a soft and smooth texture, but they could be cooked to achieve a juicier and harder meat like texture (Rousta et al., 2021).
3. Majority of the mycoproteins are tasteless, colourless, and unappealing with texture similar to meat fibre. However, with added flavouring and colouring, they can be made to resemble fish, chicken, veal, or ham flesh.
4. Mushroom health benefits and anti-tumour properties.

On a limited scale, mycoprotein powder was marketed in the UK as the first fungal food for human consumption. It was described by Newmark (1980) that the A35 strain of *Fusarium graminearum* could be processed into useful mycelia which will act as great raw material for sausages due to their organoleptic characteristics and keeping quality. By adding large quantities of proteins, raw meat could be replaced in part and easily blended to form a homogenous finely chopped material (Kumar et al., 2017). With light grey colour and distinct mushroom flavour, the developed product has the characteristics of the mushroom. A meat-like product can be produced by using edible mushrooms, gelling agents (polysaccharides that undergo gelation without reversibility), texturing agents (proteins), and flavourings. The substitution of mushrooms for texturized soy protein results in a better sensory experience for meat analogue nuggets due to an improved taste and better acceptability (Kumar & Kumar, 2011; Kumar et al., 2011).

Cultured Meat

Using in vitro growth medium, animal-derived stem cells are used to grow cultured meat. Although biologically like meat, it is not produced by sacrificing any animal. A technique derived from regenerative medicine (the field of medicine that tries to find mechanisms to obtain cells, tissues or organs) has been scaled up to produce meat through biotechnological processes and is called “cell culture”. As currently defined, it makes use of a “set of technologies for producing a range of products primarily derived obtained from livestock farming and utilizing culturing methods to produce the individual products”. Cell lines could be obtained either by genetic engineering, gene modification, otherwise by inducing spontaneous mutations using primary animal cells adapted for cell culture (Ramboer et al., 2014). Specifically designed liquid media is used to culture cells, which provide the conditions for tissue growth. Cell and tissue types will determine the type of media to be used, but the procedure needs nutrients (such as chicken embryo extract, foetal calf or horse serum, collagen, and serum-free media) (Burattini et al., 2004; Chiron et al., 2012). Additional organic and inorganic elements can also be added to the media (antibiotics, antimetabolic, carbohydrates, amino

acids, and vitamins) (Arora, 2013). Rather than a disorganized collection of muscle cells, cells require a scaffold for spreading and forming the structure that produces a tissue (for example, a muscle). Research in this field is still scarce and the components employed in the procedure based on their developmental stages. Although several companies, including Higher Steaks and Aleph Farms, already use only serum-free growing media, further study is required to lower the expenses of such processes (Stephens et al., 2018).

Microalgae

Over the past centuries, humans have eaten microalgae as a food source (Milledge, 2011). They contain several long-chain polyunsaturated fatty acids, proteins, vitamins, carotenoids, minerals, and phenolic compounds (Plaza et al., 2009). Approximately 50,000 species of microalgae are known to exist in the world. However, of these, only 30,000 species have been studied. The genus of commercial significance is *Chlorella*, *Spirulina*, *Haematococcus*, *Dunaliella*, *Botryococcus*, *Phaeodactylum*, *Porphyridium*, *Chaetoceros*, *Cryptocodinium*, *Isochrysis*, *Nannochloris*, *Schizochytrium*, *Nitzschia*, *Tetraselmis*, and *Speleochem*. Products based on *Chlorella* and *Spirulina* are the most dominant products in the current market (Sathasivam et al., 2019).

In the health food sector, microalgae can be found in tablet, capsule, and liquid forms. These ingredients are used in a variety of products, including candy, snacks, gum, wine, noodles, and beverages including breakfast cereals. A prokaryotic cyanobacterium named *Spirulina platensis* (also known as *Spirulina*) performs a crucial function in the maintenance of the planet's biosphere (Romano et al., 2011). *Spirulina* is rich in a variety of nutrients, but its high protein content is particularly interesting for the development of foods rich in protein. By using the high moisture extrusion (HME) process, meat analogues can be produced from sources such as soybean, wheat, pea, milk, egg, or fungi. A meat-like product should have similar features to meat products when it comes to texture, mouthfeel, and taste including nutritional value (Wild, 2016). By using the cooling die at the end of the extruder, the melted protein-based raw materials are aligned to produce a meat-like structure. A preliminary study, however, has suggested that HME processing of *Spirulina* results in products that lack fibrous structure (Palanisamy et al., 2019). Thus, *Spirulina* biomass should be blended with other plant proteins that are capable of texturization. Grahl et al. (2018) have investigated the organoleptic characteristics of HME-based soya meat analogues using *Spirulina* and extrusion parameters to enhance the applications of the *Spirulina* biomass and variety of meat substitutes.

Single Cell Protein

The main components of single-cell proteins are proteins, fats, carbohydrates, ash materials, water, and other elements such as potassium and phosphorus. Rapid growth of bacteria and a short generation time make bacteria a suitable candidate for SCP production (Suman et al., 2015). The bacteria can also grow on a variety of raw materials, including carbohydrate sources, such as starch and sugar, and gaseous and liquid hydrocarbons, including methane and petroleum fractions. Therefore, a number of bacteria can be used to produce large quantities of single-cell proteins. These include *Brevibacterium* (Adedayo et al., 2011), *Methylophilus methylotrophus*, *Acromobacter delvaeate*, *Acinetobacter calcoaceticus*, *Aeromonas hydrophila*, *Bacillus megaterium*, *Bacillus subtilis* (Gomashe et al., 2014), *Lactobacillus* species, *Cellulomonas* species, *Methylomonas methylotrophus*, *Pseudomonas fluorescens*, *Rhodospseudomonas capsulate*, *Flavobacterium* species, and *Thermomonospora fusca* (Dharumadurai et al., 2011).

Single-cell proteins derived from the use of bacteria have more than 80% protein content. They have low amounts of sulphur-containing amino acids and contain high levels of nucleic acids. Besides providing nutrition, proteins also serve a number of other functions in food systems (Suman et al., 2015).

Tofu

Tofu is the most commonly used meat substitute in the world and comes in block (cube/cuboid) form. Tofu or soy curd is derived from soybeans and is rich in protein and minerals. One of the many advantages of tofu production is its ease, cleanliness, convenience, and controllability. According to fresh weight basis, tofu contains about 8% of protein, 2% of carbohydrates, and 4 to 5% of lipids. Soy curd is in high demand due to its low cholesterol, high vitamin and mineral content, and presence of dietary fibre (about 1%) among other beneficial factors. It is traditionally made by coagulating protein in hot soy milk with a coagulant such as salt-induced (CaSO_4 , CaCl_2 , or MgCl_2) or acid-induced (glucono-delta-lactone (GDL), also known as gluconolactone) to yield a gel-like item (Wang et al., 2018a, b). There are still controversies over the role played by the coagulant used for tofu production in determining its quality; yet, some studies claim it is important. Soaking, crushing, and filtering the soybeans to extract the milk are the initial steps in the process. It is then heated, coagulated, broken up, pressed, and then reformed. In contrast, packaged or filled tofu involves adding coagulant to chilled soy milk and after that heating and coagulating the mixture inside an appropriate packet, without further processing. Research by Wang et al. (2018a, b) has indicated that some

people develop allergic reactions, do not like its off-flavour, and experience flatulence from milk containing isoflavones, oligosaccharides, and trypsin inhibitors, respectively. This has resulted in high demand for soy protein isolate (SPI) as a source for packaged tofu due to its superior health benefits. In addition to the coagulant used, processing conditions, coagulant concentration employed, and two storage proteins Glycinin (11S) and β -conglycinin (7S) are also the significant factors in tofu making (Kumar et al., 2020).

Seitan

Seitan is also a popular vegetarian meat alternative manufactured from wheat gluten commonly called “wheat meat” or “wheat protein”. The chewy mass consisting of protein components gliadin and glutenin are obtained after rinsing wheat dough to remove the starch and bran components. Vegetarian sausages, burgers, nuggets, schnitzels, and minced meat are made using seitan as it is a simple and cost-effective raw material. Additionally, the wheat crop is indigenous to a large number of countries around the world, and therefore, seitan can be produced globally. Since seitan is a stringy substance, its consistency is remarkably similar to that of meat (Schmidinger, 2012).

A variety of ways can be used to season and prepare seitan-based food products. Reducing agents, proteases, or glutathione could be added to the elastic wheat gluten in order to cut the peptide linkages and obtain desired physical properties (Schmidinger, 2012). Moisture content is adjusted between 40 to 80% (w/w), and then the mix is extruded into a pipe/cylindrical shape, or sheets through the use of a nozzle and formed into continuous sheets of thickness 4 to 20 mm and width 100 to 1000 mm. During the stretching process, the sheet is extended at least 2.5 folds, which provides directionality to the fibre structure. The resulting sheet is elevated in airspace that has approximately a relative humidity of 75% and a temperature of 75 to 120 °C for the next heating process (Joshi & Kumar, 2015).

Textured Soy Protein/Soy-Meat

In food industries, vegetable proteins (VPs) are used to increase the protein consumption and essential amino acids thereby reducing the consumption of saturated fat and cholesterol. Textured vegetable protein (TVP), which is a fibrous, insoluble, and porous form of vegetable protein, is made from a variety of sources like legumes (protein content may vary from 25 to 50%), nuts, and oilseeds, and out of these, due to its high protein content soybean makes it an excellent raw material for producing meat substitutes or meat analogues (Kumar et al., 2020).

Textured soy protein (TSP) or soy-meat is produced when defatted soy flour is processed by concentrating, isolating, and extruding protein to provide a chewier texture and a better flavour than meat or seafood. Protein content in soy-meat is about 50% that decreases when rehydrated. The TVP technology was launched in the USA and introduced successfully into European markets in late 1960s. Currently, vegetarians and health-conscious consumers consider soy-meat an economical alternative to meat (Alamu & Busie, 2019). By replacing 30% of meat with TSP, many meat products could still be produced without affecting the quality of the product. There is no significant change in the sensory characteristics of samosa stuffing in certain regions of the world where beef is replaced with granules of defatted soy flour by around 50%. TSP is created by the use of ‘hot extrusion’ technique which produces high protein nuggets, chunks, etc. by using different dyes. The consumption of soy-meat is rapidly growing around the world today, especially in developed nations. As the soy-meat is an excellent protein source, it is often referred to as a “poor man’s food” by those in under-developed countries or low-income families (Alamu & Busie, 2019).

Benefits of Plant-Based Meat Analogues

The Ecological Impacts of Plant-Based Meat vs. Meat Consumption

As a result of environmental degradation and climate change, plant vs. meat debate takes on new dimensions, both of which are harmful to human health and must be taken into account when recommending diets for livestock or humans. Providing nutrients (e.g., folate, manganese, thiamine, copper, and β -carotene) from plant-based foods may have a smaller environmental footprint (e.g., emitting fewer greenhouse gasses) than getting similar nutrients from animal-based foods (Eshel et al., 2019). However, some experts suggest that omnivorous diets and animal foods have a higher carbon footprint when compared to carefully selected plant-based diets that provide same quantities of protein, iron, and vitamin A (González et al., 2011). These comparisons, however, ignore the fact that plant sources for these nutrients have a reduced bioavailability and bioaccessibility (van-Vliet et al., 2015).

As a result of putting more pressure on the environment and ecology than their plant counterparts, animal foods become a cause of concern despite their importance as foods that are more readily available to satisfy our needs for specific nutrients (Drewnowski et al., 2015; Tessari et al., 2016). Many people choose plant-based meat alternatives over animal meat because of their lower carbon footprint. According to the recent life-cycle

analysis (LCA), the Beyond Burger® and the Impossible™ Burger have a lower carbon footprint (+ 3.2 and 3.5 kg CO₂-eq emissions/per kg product, respectively) than US beef fed on mixed rations in feedlots that ranges from + 10.2 to + 48.5 kg CO₂-eq per kg product, varying on the model employed, the geographical region where the cattle are raised, and the inclusion of GHGE possibilities of retail, transportation, restaurant or at home use, and end-of-life cycles (Heller & Keoleian, 2018; van Vliet et al., 2020).

Environmental Benefits

Food production is one of the most environmental damaging operations that account for 30% of global greenhouse gas (GHG) emissions and 70–85% of global water footprint (Smetana et al., 2015). With the increasing demand for food, as well as the impacts of meat production, consumer awareness has increased. Alongside food security, deforestation, pollution, and environmental and socioeconomic issues have become extremely important. On a larger scale, consumers are seeking out sustainable foods and environment friendly methods of production, leading them to become vegetarians or reduce meat consumption on a weekly basis (Dagevos & Voordouw, 2013). As a result of lesser meat consumption, less water and feed are consumed, and the associated emissions are reduced. Thus, plant-based diets possess same nutritional and sensory attributes as meat-based diets that offer multiple advantages after being used.

Plant-based meat alternatives have been found to have a lesser environmental impact compared to animal meat. Several plant-based meat substitutes are reported as nutritious as animal meat, and have less environmental impact than animal meat in some cases (Sturtewagen et al., 2016; Van-Mierlo et al., 2017). Researchers have found that combining environmental constraints and nutrition considerations with modelling techniques (such as linear programming) caused meat products to be reduced or even eliminated, and thus decrease its environmental impact significantly. Additionally, Tyszler et al. (2014) investigated the possibility of varied combinations of foods that help to compensate for the shortage of certain essential amino acids in a strict vegan diet. Despite this, many studies have revealed that a complete vegan diet combined with only plant-based products is not the one having the least effect on the environment. However, the amount of meat or other animal-based items consumed must be kept to a minimum. Therefore, incorporating plant-based meat alternatives into our daily diet can be beneficial for health, cost-effective, and environment friendly approach (Kyriakopoulou et al., 2019).

Nutritional and Accessory Benefits of Meat Alternatives

Meat is consumed primarily due to its high nutritional value. It is therefore vital to develop plant-based meat alternatives that meet the nutritional requirements as of traditional meats (Asgar et al., 2010). It is generally assumed that plant protein has less nutritional value than animal protein as it lacks lysine, methionine, and cysteine (Joshi & Kumar, 2015). Thus, plant proteins have low bioavailability.

The most common sources of plant proteins to produce plant-based meat substitutes are wheat gluten and soybean proteins due to their high nutritional values and functionality (Asgar et al., 2010). One of the most essential crops around the world is wheat which contains 8–17.5% protein. Gluten (subdivided into gliadin and glutenin) is produced during the washing of wheat flour with water, which is considered as “GRAS” (generally recognized as safe) commodity. An increase in temperature of 85 °C can coagulate gluten, leading to gel formation without losing their structural order (Lee et al., 2020).

Gluten can also be used as a source of plant protein to obtain meat analogues due to its cohesive blend with other ingredients. Protein is also obtained from legumes such as clover, peas, and alfalfa, as well as soybeans. As good sources of protein and having economic benefits, plant proteins have recently attracted the attention of consumers. Malav et al. (2015) revealed that soybeans contain 35 to 40% high-quality proteins, 15 to 20% fats, and 30% carbohydrates with Fe, Ca, and Zn minerals. Because of its great rehydration ability, oil absorption, emulsification, and water absorption properties, soybean protein can be utilised as a meat alternative (Liu et al., 2017).

Several plant-based meat alternatives currently found in the market appear to provide our diets with enough protein. A study conducted by Bohrer (2019) examined the nutritional content of four varieties of conventional meat and plant-based meat substitutes (beef burgers, pork ham, beef meatballs, and chicken nuggets) available in the market. They observed that a beef patty inside burger contains 23.33 g of protein, in comparison to meat analogue patty that weighs approximately 19.46 g. The plant-based meat alternative, however, is lower in cholesterol and higher in fibre, which makes them attractive to consumers. Also, the performance of different products (pork ham, beef meatballs, and chicken nuggets) was same (Bohrer, 2019). Hence, plant-based meat analogues are likely to be better meat alternatives in terms of nutrition, especially protein content and are also healthy. Many religious and ethical beliefs prevent consumers from eating meat or meat products, which means these products (plant-based meat analogues) will be of benefit to such consumers. Among the various meat alternatives, with a rapidly expanding market for halal and kosher food

items as well as growing interest in animal welfare, it is likely that plant-based meat replacements are projected to be in high demand in the future (Lee et al., 2020).

Methods for Development of Plant-Based Meat Analogues

Takeover by plant-based alternatives in our everyday diet has begun decades prior, be it for milk substitutes or meat substitutes. Numerous foods that we eat up today are made up of plant protein and had been utilized as a plant-based protein source for a long time for replacing and reducing the rate of consumption of meat and meat-developed products (Pearson, 2012). The production of meat analogues or substitutes in general mainly follows four crucial steps:

1. Protein crops with superior quality are recognised and cultivated across the globe.
2. Crops undergo different processing techniques to obtain protein ingredients such as protein concentrates and isolates.
3. These protein components are thereby combined and processed into texturized intermediate items from which meat analogues are created.
4. Products are dispersed between end-users by retail and various food delivery methods.

Depending on how the fibrous morphology is produced, different structuring techniques are used. The different types

of meat analogue production technologies along with their associated advantages are given in Table 3. An individual structural element can be designed and then assembled into an entire product using a bottom-up approach (Heuvel et al., 2013). By growing filamentous fungi or by making proteins that are similar to the myofibrils, or by creating fibres by wet-spinning or electrospinning, structural elements can be produced. It is recommended that each element is aligned prior to assembly to create the same structural anisotropy as meat (Heuvel et al., 2013). It is possible to get anisotropic properties of animal muscle by using scaffolds that let cells grow in one direction. In the other strategy, the individual structural elements must be aligned and assembled using directional processing. This is known as the bottom-up approach.

Others emulate structure on large scale through the use of top-down strategy. These structures resemble the meat structure in their arrangement but not in other attributes. Biopolymer(s) are formed by aggregating or placing them in an anisotropic field of force. Ice crystals are aligned as they grow in unidirectional freezing. Shear forces are used in some of the techniques to create anisotropy (Dekkers et al., 2018). Taking into account two of the major components, such as water and biopolymer, these processes can be simplified. Consequently, the continuous and dispersed phases are separate phases since they do not mix into each other. The fibrous structure in products is generated by a continuous (mostly protein) phase which is disrupted by filaments at the matrix-breaking stage: filaments which are dispersed, deformed, and weak. Materials get affected by this when

Table 3 Types of meat analogue production technology with their associated advantages

Technology	Type	Advantages
Extrusion	Top-down strategy	-Higher productivity and lower costs -Highly versatile -Energy efficient process -Destruction or conversion of anti-nutritional factors -Enhancement of protein digestibility
High-temperature induced shearing	Top-down strategy	-Cost-effective -Produce defined fibrous structure
Wet-spinning	Bottom-up strategy	-Produce defined fibrous protein products
Electrospinning	Bottom-up strategy	-Cost-effective and scalable approach -Production of very thin fibrils
Freeze structuring	Top-down strategy	-Modulation of textural properties of plant proteins
Mixing plant proteins and hydrocolloids	Top-down strategy	-Formation of fibrous structure that can be modulated
Bioprinting (3D printing technology)	Top-down strategy	-Enable the design of products with texture similar to muscle fibres -Tailor the nutritional content of the product
In vitro meat: cell culturing	Bottom-up strategy	-Less energy consumption than conventionally produced meat -Low emissions of greenhouse gases -Lower land and water use
Mycoprotein	Bottom-up strategy	-High in protein and fibre, and low in fat, cholesterol, sodium, and sugar -Low carbon and water footprint

Source: Boukid (2020)

they are mechanically processed or modified (Dekkers et al., 2018).

Techniques that Follow a Top-Down Approach

Extrusion

Extrusion is the most coveted technique to transform plant-based materials into fibrous products. Two classes of extrusion have been identified: one is low-moisture extrusion and another one is high-moisture extrusion. When talking about low-moisture extrusion, flours, or concentrates are processed into textured vegetable proteins (TVP) using mechanical operations. The products made in such a way are dry and slightly expanded that are moisturized afterwards. In case of high moisture extrusion, fibrous products are generated with moisture contents above 50% of their weight. The proteins are softened inside the barrel using a blend of heating, hydration and mechanical deformation. This protein “melt” gets lined up by the (inhomogeneous) laminar flow upon its drift into the die and gets cooled down to fend off expansion. While extrusion is the most widely applied technology when production of meat analogues is the goal, it is also comparatively energy consuming and a very complex process which does not offer greater control over the final product morphology.

Zahari et al. (2020) used a laboratory co-rotating twin-screw extruder having a fixed screw configuration with a screw diameter of 20 mm to process the high moisture meat analogue (HMMA) products. The extruder was having 4 different temperature zones in the barrel, and they were kept at 40, 60, 80, and 100 °C throughout the experiment. Concentration of hemp protein concentrate (HPC), target moisture content, temperature, and screw speed were the chosen parameters considered. It was found in the preliminary experimentation that the screw speed of 800 rpm produced the most acceptable texture of HMMA products for all HPC mixture formulations. But for SPI alone formulation, only soy protein isolate could be extruded using 500 rpm at 70% and 75% of the targeted moisture content (Zahari et al., 2020). Hence, eleven formulations were executed for the given experiment. The moisture content of the raw materials was used to calculate the solid dosing (0.8–1.1 kg/h) and liquid dosing (1.88–2.36 kg/h), while software was used to set the target moisture content of the product and the total mass flow (3 kg/h). The cooked blend was forced to pass through a long flat shaped cooling die with measurements of 25 × 7 × 300 mm (W × H × L), and a water jet was used to cool it down from outside. The cooling process increases the viscosity of the mixture, thus bringing about shear which gives rise to a layered and fibrous structure. Extruded products were cut by manual means after emerging from the end of the cooling die and made to cool at ambient conditions

before transferring them into a sealed plastic bag and kept frozen at – 18 °C for further analysis.

In another methodology, Rehrah et al., (2009) mixed peanut protein concentrate (93% protein) with defatted peanut flour (55% protein) to get a material with protein content in range of about 60–80%. The moisture of the mixture was adjusted to about 40–90% (w/w) through addition of water into the dry ingredients and mixed using a kitchen mixing aid. After 20 min of mixing, the samples were sealed in plastic bags and stored at ambient temperature for 10 h to bring them into equilibrium before extrusion.

Extrusion was done using a single-screw laboratory scale extruder with barrel configuration as 380 mm long and 19 mm inside diameter (20:1 L/D ratio). Manual feeding of the peanut paste samples was done through a standard bin feed hopper using a rod die (12-mm diameter) with 5-mm nozzle. There were three temperature-controlled zones in the barrel in which heating was done electrically (Rehrah et al., 2009). Temperature settings were put up at 80 °C for the extruder barrel, with settings for variable temperature at (100–190 °C), and ambient conditions for the first (feeding zone), second, and third zone, respectively. Based on extrudate quality, the optimal parameters for extrusion were determined using response surface methodology, where 60–65% protein, 50–55% moisture, 160–165 °C, and 80–90 rpm were utilized for the production of extrudates. Immediate cutting of the extruded samples (35% moisture) was done as they left the die outlet and collected for analysis of their textural properties (Rehrah et al., 2009).

Freeze Structuring

Texture or fibrous structure is one of the most problematic things in relation to the making of meat analogue, and the same could be bettered by improving the process conditions or by selecting the protein sources from plants (Palanisamy et al., 2018). Different established methods could be utilized for creating the fibrous structure of plant protein ingredients. Freeze structuring consists of freezing a protein emulsion in order to create a distinct fibrous structure. A porous and fibrous microstructure could be generated by the subsequent removal of ice crystals, which is similar to the muscles of animal meat consisting of a number of parallel and highly connected sheet-like proteins (Dekkers et al., 2018). The protein source used for the manufacture of meat analogues is mainly responsible for the formation of fibrous structures using the freeze structuring technique. Hydration and solubility, and gelation and texture formation are some of the distinct structure–function parameters that control the ultimate structure of the meat analogue, and they could be different for different proteins (Meade et al., 2005). Pea, soy, wheat, and fungi protein are some examples of plant proteins incorporated in meat analogues (Joshi & Kumar, 2015).

Yuliarti et al. (2021) obtained pea protein isolate along with wheat protein, all-purpose flour, potato starch, methylcellulose (MC), vegetable oil (blend of palm, peanut, and sesame oil), baking powder, and chicken flavouring powder. A total of 5 different ratios of the composites were used to prepare the plant-based nugget (PPN or pea protein nuggets). Calcium chloride and baking powder were added into each composite with the aim of improving the protein water binding capacity and for generating air cells in the meat analogue dough subsequently, thereby improving the fibrous structure of the analogues. Protein and methylcellulose emulsions were used separately for the preparation of the analogue and then both were combined and mixed for a few minutes. A food processor was used to form the emulsion by mixing the ingredients at a low speed for few minutes. The resulting PPN analogue batter was then moulded and steamed. The PPN samples were coated with batter (prepared by combining flour and DI water in ratio of 1:2) following the steaming process and deep-fried on both sides. Immediate freezing of the analogues followed next. The analogues were baked before they were used for any analysis purpose.

Shear Cell Technology

In recent years, a new technique has been developed based on flow-induced structuring (Peighambardoust et al., 2004). The shear cell was developed for this purpose and is based on a cone–cone geometry and follows the principal of cone–plate rheometer. The cone on the top side of the device remains stationary while the cone on the bottom side rotates. Both cones can be heated and cooled in an oil bath. In contrast with extrusion, the deformation inside the device is more precise and constant during processing. Proteins are aligned by simple shear and heat, which creates fibrous structure (Manski et al., 2008). Shear cells have been shown to be able to fibrillize even dairy proteins (calcium caseinate). Because the radius of the shear cell increases with distance between the cones, the shear rate for the protein sample is not constant throughout the volume. This configuration is also limited in scalability, so it can only be used for lab-scale testing. By simply increasing the diameter and length of the cylinders, the Couette cell design can allow for greater thickness and capacity (Manski et al., 2007). In the future, the Couette cell will also be potentially able to operate continuously.

The device was originally developed to study the behaviour of dough under simple shear flow and was not optimized for operation at elevated temperatures. The Couette cell was used to shear a blend of plant proteins after several design upgrades were made to enhance heat management and material handling of the original device. An experimental study was conducted to demonstrate the process concept, including analysis of process temperatures, process times,

and rotations per minute (RPMs) of the inner cylinder. SEM, texture analysis, visual inspection, and tensile stress were all used to characterize the products. The experiment was not designed to optimize the operating conditions, but rather to demonstrate the feasibility of making anisotropic plant protein–based structures with a Couette cell under mild process conditions.

Krintiras et al. (2015) used a blend of soy protein isolate (SPI) and vital wheat gluten (WG) along with sodium chloride. The mixture had a dry matter content of 31%, with SPI–gluten ratio of 3.3:1. Then, salt was added to demi-water in a flask, and shaken to completely dissolve it. The salt solution was then added to the SPI in a glass beaker, and the mixture was manually mixed with spatula in a glass beaker and covered to prevent moisture from escaping. The mixture was left to rest for some time, as preliminary works reported that pre-humidification for some time enhances protein structuring. Adding gluten lastly, followed by quick mixing prevented the mixture from lumping together. The mixture was ready to be processed in the Couette cell. The Couette cell was filled with material using a transparent (caulking) filling gun container, a funnel, and a pounder. After filling, the mixture could be effectively deaerated. A filling tube which was screwed into the filling hole allows it to fill the Couette cell from the side. A thermocouple was attached to the screw plug that seals the filling hole when the space between the cylinders (shearing zone) has been completely filled, and the mixture is tightly packed. Afterward, the experiment was initiated quickly. Fill-up time was determined by checking the period between the end of the mixing process and the beginning of the experiment. According to an estimate of the characteristic heat conduction timescale for the process, no preheating of the mixture was necessary prior to the experiment (Krintiras et al., 2015). To cool the material inside the Couette cell, the oil circulation from the hot oil bath was shut off, and the cold oil bath was turned on at the end of each experiment. In the next step, the lid and outer cylinder were removed, and the sample was cut vertically, resulting in a rectangular slab. As soon as the sample was removed from the Couette cell, its weight was measured. Weighted samples were packed in sealable bags and placed in the freezer. Samples were recorded for temperature and relative humidity.

Dekkers et al. (2016) used soy protein isolate, pectin, sodium chloride, and glutaraldehyde. With a constant dry matter, shear-induced structure formation in pectin/SPI blends was investigated. Thus, the total weight was a combination of NaCl, polymers, and demi-water. Pectin and SPI were added in increasing amounts by removing parts of SPI and replacing them with pectin. In order to mix the ingredients, demineralized water was first dissolved in salts, and then SPI was added to the salt solution. Dispersion of protein was achieved through hydration. To the dispersion, pectin

was added and thoroughly mixed. It was found that hydrating protein and pectin in this order produced the most distinct fibrous structures, and therefore, it was used (Dekkers et al., 2016).

To produce anisotropic samples, the pectin/SPI blends were consolidated in a high temperature shear cell. The shear cell was developed from previously reported equipment and constructed in-house. Adaptations were made to the device to allow it to operate at high temperatures. Essentially, there were two cones: a rotating bottom cone and a stationary top cone. A thermocouple measured the temperature inside the cones while the cones were heated and cooled in an oil bath. Rotation speed was controlled with a Haake drive. Three different temperatures were used to heat the SPI and pectin/SPI blends. The blends were placed in the shear cell preheated to the set temperature and sheared for some time. The samples that were not sheared were simply heated inside the shear cell. Following the cooling process, the samples were removed from the shear cell, prepared, and were frozen afterwards (Dekkers et al., 2016).

3D Printing

Fused deposition modelling (FDM), also known as 3-D printing, is a major type of additive manufacturing (AM) that also includes photographic and stereolithographic methods (Severini et al., 2018). There are a variety of processes that can be used for 3D printing of meat analogues. These include extrusion, inkjet printing, binder jetting, and bioprinting. In order to form structures from fibrous meat materials, meat analogues and their products are 3D printed using an extrusion process (Min et al., 2019). Despite other methods that are being developed for 3D printing of meat products with the desired design, the extrusion process is the most promising. This method involves layer-by-layer extrusion of three-dimensional geometric structures via a nozzle. Further, extrusion usually involves semisolid pastes such as dough, chocolate, or meat purees. Liquid materials are dispensed in the form of liquid foods in inkjet printing by using a continuous jet or drop-on-demand method that have low viscosity. Powders (e.g., cocoa and sugar) are commonly used to form 3D structures through binder jetting. In addition to bioprinting, the usual three methods are also utilized: jetting, lasers, and extrusion (Min et al., 2019). Using an inkjet printer head situated over the print bed, bio-ink droplets are formed electrostatically or piezoelectrically by electrostatic or piezoelectric forces. Cells are deposited on matrices using lasers in laser methods. The last step in the extrusion process involves the release of the bio-ink through a syringe and piston system, which extrudes the material layer by layer. There has been increasing interest in bioprinting due to its potential application in tissue engineering (Wang et al., 2018a, b).

In particular, plant-based meat alternatives are being developed by start-up ventures such as Beyond Burger and Impossible Foods. Even though Impossible Foods uses various plant-based ingredients, soybean leghaemoglobin is the primary ingredient responsible for the meat's distinctive colour, as it contains the pigment protein myoglobin. As opposed to Impossible Foods, Beyond Burger uses beet juice extract, whereas, another start-up uses tomato paste (Wi et al., 2020). In addition, other natural pigments such as red peppers, paprika, annatto, and red rice, can also be used. As for 3D printing, NOVAMEAT (Barcelona, Spain) claims to have manufactured beef and chicken using this technology. Several ingredients such as pea, seaweed, and beetroot juice were reportedly used to print steak by this company in 2018. The organoleptic study of the product indicated that they were similar to regular beef steaks, with its fibres having diameters in the range of 100 to 500 μ . The cost of fabrication of a 50-g steak was \$1.50 USD, showing that these processes are cost-effective (Youssef & Barbut, 2011). A majority of the obstacles in the development of 3D meat analogues are lack of essential amino acids, the need to texturize plant proteins, and the need to incorporate non-proteinaceous ingredients. In addition, plant-based analogues consist mostly of sausages, patties, and mince. There seems to be a challenge in developing steaks from plant-based materials. In addition, mushrooms, another ingredient which resembles meat, have also been used in 3D printing snack recipes. Fibre-rich foods pose a challenge when they come to printing because the complex network of fibres can clog printer nozzles as a result of aggregation (Yuliarti et al., 2021).

Techniques that Follow a Bottom-up Approach

Cultured Meat

The first-time animal organs were alive out of the animal body was when animal tissue was grown in vitro and beyond the confines of the vertebrate body for some time, and this was done with the help of artificial oxygenation and nutrient supply. A similar technique has been described as being employed to grow meat in Winston Churchill's book (Carrel & Lindbergh, 1935). It was not until a couple of decades later that cell and tissue culture became a reality. As cell biology and tissue engineering evolved in medical research during the past three decades, human tissue could be grown on a small to intermediate scale. After scientists identified the muscle-specific stem cells (satellite cells) and found that these cells can differentiate into muscle fibres and self-organize, it became feasible to culture muscle on a large scale.

In theory, it is quite easy to grow meat (Vandenburg et al., 1996). One begins with a small piece of fresh muscle,

which is obtained by performing muscle biopsy on a living animal. The satellite cells in the muscle fibres are liberated from their subsarcolemmal position through a specific combination of mechanical and enzymatic disruption. After a period of 7–8 weeks of culturing, the cells are found to divide in up to 50 population doublings using traditional culturing methods. However, the cells are kept at high serum concentrations of 300-mL per litre. As soon as sufficient numbers of cells have been obtained, they are divided into 1.5 million portions, and each batch is covered with collagen/matrigel gel that is displayed in a culture dish surrounded by an agarose gel hub at the centre. The muscle fibres self-organize themselves over the following days into a donut-shaped muscle fibre with a diameter of 1 mm. Muscle maturation and protein synthesis are stimulated by the tension that is created in the ring structure by muscle contractions. It takes 3 weeks to harvest the muscle fibre (Boonen et al., 2010). Ten thousand of these muscle strips were used to prepare and cook the 85-g hamburger that was presented, cooked, and tasted on 5 August 2013 in London (Post, 2014).

Mycoproteins

Several meat analogues have been developed using *Fusarium venenatum* since the mid-1980s. These are marketed as Quorn products. A continuous fermentation process is used to create the fungus in bioreactors. In the bioreactor, temperatures and pH levels should be tightly controlled, as they are critical factors in the production of the fungus (Dekkers et al., 2018). In order for the RNA to diffuse out of the cells after fermentation, it must be degraded into monomers by heat treatment. As a result of centrifugation and heating of residual biomass, a paste-like product is obtained with a 20% solid content (Wiebe, 2004). Despite this centrifugation step, filamentous fungi are not ordered; therefore, they must undergo a number of additional process steps, such as forming, steaming, chilling, and texturizing to become fibrous (Smetana et al., 2015).

Effects of Methods on Properties of Plant-Based Meat Analogues

Effects of Extrusion

Cutting Force

Hardness and fibrous structures are indirectly measured by cutting force based on degree of texturization or hardness. With increasing protein concentration, the cutting force decreased significantly, indicating that the formation of fibrous structures is diminished. The cutting force

increased as the temperature increased, but only at higher temperatures. Perhaps higher temperatures result in more denaturation of proteins. An increase in screw speed was responsible for a significant increase in the cutting force of meat analogues. The effect of extrusion temperature, and screw speed on cutting force is consistent with the studies of Grahl et al. (2018). The sensory acceptance of meat analogues can be influenced by both the hardness and the level of fibrousness/texturization.

Product Expansion Ratio

It is an indicator of the degree of puffing and one of the most important physical characteristics of the extrudate. Barrel temperature shows a significant difference ($p < 0.05$) in the lateral expansion of the meat substitute. Bhattacharya (1997) explained that a high input of thermal energy combined with a long residence time leads to a high level of superheated steam, which produces flashy and porous structures as the cell expands. The pressure drops suddenly from high to atmospheric when extrusion cooked melt exits the die. During this pressure drop, internal moisture and the water vapour pressure are released which subsequently nucleate to form bubbles in the molten extrudate and allow it to expand (Arhaliass et al., 2003).

Cooking Yield

The amount of water absorbed during cooking also affects sensory properties, such as juiciness. By increasing the temperature and screw speed, and decreasing the water feed and protein concentration, resulted in hard products exhibiting higher cutting force and cooking yield. Based upon this, it can be said that hard structures are better at holding water without dripping than soft structures. It can be speculated that increasing screw speed and temperature and decreasing water feed may induce more insoluble protein aggregates during the extrusion process. Researchers found that the better water entrapment between protein networks resulted in higher water holding capacity in higher protein concentrations, which could account for the higher cooking yield in extrudates (Palanisamy et al., 2019; Vardhanabhuti et al., 2001).

In Vitro Protein Digestibility

It has been shown that extrusion of proteins can change their secondary, tertiary, and quaternary structures, which then provides a better access for proteolytic enzymes and ultimately improves the protein digestibility (PD) (Ruiz-Ruiz et al., 2008). Spirulina incorporated extrusion mixtures showed decreased PD in comparison to raw extrusion mixtures containing lupin proteins. Compared to raw

extrusion mixtures, all extrudates except the one with 50% *Spirulina* added exhibited a higher PD. The composition of the cell walls of the microalgae biomass may contribute to the decrease in PD. Despite the thin cell walls of *Spirulina* (40–60 nm) (Kose et al., 2017), they were thought to have caused the negative effects in contrast to pure lupin protein mixtures and extrudates without the presence of cell walls. PD did not change much after changing extrusion temperature, water feed, and screw speed.

Bulk Density

An increase in screw speed produces a denser extrudate, while an increase in barrel temperature produces a low density extrudate. Study by some researchers confirmed that higher barrel temperatures cause more superheating of water in the extruder, which causes bubbles to form and also a decrease in melt viscosity, which results in less density. The increase in bulk density caused by increased screw speed may be due to increased temperature effect on extruded melt under a high-shear environment (high screw speed). There is a high likelihood that this will result in a longer gelatinization process and a higher extrusion rate, which is also in agreement with the report of Li et al. (2005).

Water Absorption Index

It is the result of starch being dispersed in excess water due to gelatinization and extrusion-induced fragmentation, which increases the dispersion. It measures the volume that starch granules occupy after swelling in excess water (Altan et al., 2008). A major effect of extrusion on starch components of foods is gelatinization, the conversion of raw starch into a cooked and digestible material through water and heat (Ding et al., 2006). Increasing temperature and screw speed can lead to a reduction in WAI of extrudate, which is a measure of juiciness and moisture of the product after hydration. The water absorption index decreases significantly when these factors are considered. WAI decreased with increasing temperature probably due to degradation or decomposition of starch (Pelembé et al., 2002).

Oil Absorption Index

The oil holding capacity (OHC) of proteins is important to understand since this affects their emulsifying properties (Biswas & Sit, 2020). Accordingly, the oil absorption index of the extrudates increases with higher barrel temperatures and decreases with higher screw speeds. The increased oil absorption is possibly due to the degradation of starch in the extrudate due to high thermal energy input (Omohimi et al., 2014).

Swelling Power

It is its ability to absorb water in such a way that its starch granules expand in size, which is indicative of how much of the internal structure of meat analogues has been subjected to the action of water (Ruales et al., 1993). Increase in barrel temperature tended to increase swelling power while increases in screw speed tended to decrease it.

Shear Strength

The shear strength, which measures the mechanical strength of fibre extrudates, significantly decreases as the temperature and the protein content decreases. As a result of low shear resistance and brittleness, extrudates with low-protein levels disintegrated during texture testing. Moisture content and temperature both have an important impact on the texture of the meat analogues. The hardness of the meat analogues increased with decreasing moisture content (Lin et al., 2000). Extrudates with a higher moisture content were more pliable and could not be ruptured easily, probably because the number of protein–protein interactions decreased. However, due to a combined action of high moisture and temperature, peanut extrudates had a higher shear force. According to Aguilera et al. (1980), texturized peanut protein (TPP) had a higher shear strength than texturized soya protein (TSP) as well as a higher juiciness and better shape retention than TSP. Studies found that temperature did not significantly affect the texture of soy protein isolate when extruded at moisture levels exceeding 70% (Rehrah et al., 2009).

Effect of Shear Cell Technology

Structure Formation

As with soybeans, gluten is expected to form separate phases that align. In one study, both SPI and gluten were sheared independently and in combination (Grabowska et al., 2014). It was found that SPI alone could not produce fibres, but gluten alone at 30% (w/w) could produce fibrous structures. In addition, the sheared SPI-gluten blend produced anisotropic structures, and the possible mechanism of structure formation was attributed to the existence of two distinct phases (SPI and gluten) and an interplay between them. Likewise, Manski et al. (2008) showed that highly viscous systems tend to form structurally robust structures under simple shear. As a result of high viscosity, anisotropic structures are favoured since shear stress is evenly distributed across all phases. Moreover, when these anisotropic structures are heated, they become solid (Krintiras et al., 2015).

Effect on Properties

Protein isolates must be present in sufficiently high concentration to form a gel (soft solid) (Renkema, 2001). Despite having sufficient water, higher concentrations were not possible due to the lack of plasticity caused by high moisture availability. An analysis of the mechanical properties of samples with different concentration of protein isolate gave no evident anisotropy as the strain and tensile stress were same for parallel and perpendicular samples. With the highest concentration, measurements became less reproducible, indicating a larger degree of inhomogeneity. The crumbled 30% (w/w) material ceases to form a cohesive matrix, and therefore, the result is consistent (Grabowska et al., 2014).

Effect on Texture

Over the duration of the process, fibres can be observed as it does not seem to be a critical factor for the fibre formation. During prolonged shearing, Manski et al. (2008) found that damage to the structure resulted in a sharp torque increase in the torque curve trend (Manski et al., 2008). Both stress and strain anisotropy indices decreased in the experiments after some time, which can indicate that the newly created structures have been damaged. Visual inspection or a trend change in the torque curves did not reveal any damage to the samples after some duration of processing (Krintiras et al., 2015).

Effect on Microstructure

Microfibers of finer sizes formed thick bundles of varying particle sizes. A study by Manski et al. (2007) determined that fibres were aligned towards the flow direction. A possible explanation for the characteristic round cavities scattered throughout the domain is that they originally contained air or water. Upon closer inspection, gluten fibres with their characteristic needle-like structures were observed (Krintiras et al., 2014, 2015, 2016; Zaidel et al., 2008).

Effect of Freeze Structuring

Texture

In comparison with the faster carbon dioxide method, plate freezing might result in the formation of larger crystals, due to a slower cooling rate. This led to the formation of relatively larger voids between the protein fibres in the proteinaceous slurry. A slow cooling process leading to the formation of large ice crystals should also result in relatively thick fibres in the proteinaceous slurry (Lawrence

et al., 1986). Despite this, the thickness of the protein fibres was not significantly different between the two methods employed (Jantawat & Rojanakorn, 1996).

Microstructure

Liquid nitrogen quickly frozen the sample to form a highly porous, randomly oriented protein mass. A number of ice nuclei were present at these small voids as a result of the rapid freezing (Fennema, 1973). The fibrous protein mass exhibited a very different orientation on a macro level following unidirectional freezing. Protein sheet structures are homogeneous in their mass according to a cross-section analysis. A visible difference in appearance exists between the freeze-texturized samples and the original material, demonstrating that freezing changes the level of organization in proteins. During the thawing process at room temperature and the subsequent heat setting, the fiberization, extended crosslinking, and mechanical compression by ice crystals were able to provide the protein with considerable cohesive forces (Lawrence & Jelen, 1982). It appears that freeze texturized proteins have two distinct levels of orientation: parallel-oriented protein sheets and entangled fibres that are arranged randomly within these protein sheets, a feature that distinguishes freeze texturization from other methods for texturing proteins (Consolacion & Jelen, 1986).

Effect of 3D Printing

Effect on Microstructure

Reduced-fat meat analogues containing biosurfactants produced objects with a high level of porosity. The structure and orientation of spaces were uniform. Additionally, their micrographs showed a high solid packing density with fewer void spaces (Shahbazi et al., 2021b).

Effect on Structure

3D printed objects exhibited different levels of hardness depending on their composition and structure, which were defined by the elastic modulus, yield stress, and tensile strength (Shahbazi et al., 2021a). Formulations containing EHEC, DS inulin, OSA starch, and acetylated starch displayed a significantly higher degree of hardness ($P < 0.05$) (Shahbazi et al., 2021b).

Effect on Protein Solubility

An increase in the amount of solubilized proteins was observed when urea, DTT, or SDS are mixed with PB, i.e., PBU, PBD, and PBS solvents, respectively. Hydrogen bonds are more important in the formation of the protein networks

as witnessed by the higher values of protein solubilized with PBU as compared with PBD and PBS. Moreover, PBS and PBD solubilized proteins were of lower significance due to hydrophobic interactions and disulphide bonds (Shahbazi et al., 2021b).

Effect of Cell Culturing

Colour

Muscle fibres currently being formed are yellow, not pink or red. Myoglobin, which is responsible for most of the red colour of meat, is suppressed when cells are cultured in ambient oxygen. Many studies have shown that bovine muscle fibres can be cultured under low oxygen conditions to increase the expression of myoglobin (Kanatous & Mammen, 2010). According to experiments performed at Maastricht University on bovine myoblasts, these observations were confirmed (unpublished test results). Metabolic studies on the incorporation of iron and heme have not yet been undertaken. Meat's taste is likely also influenced by heme and iron (Post & Hocquette, 2017).

Flavour

As a result of complex compositions of proteins, sugars, and aromatic compounds, meat has a unique flavour. Our assumption is that the biochemical composition of the tissues can be similar, since myoblasts are essentially the same as the ones that produce livestock beef. That would also mean that the taste would be the same (Vettor et al., 2009). Muscle cells and some fibroblasts were the only ingredients in the proof-of-concept hamburger. As a result, meat fats lacked the aroma compounds normally found in meat. Adipose tissue must be added to the product in order to enhance flavour, as well as provide nutritional value. Cells derived from mesenchymal stem cells, stem cells from adipose tissues, and possibly even myoblasts can be used to make adipose tissue (Post & Hocquette, 2017).

Texture

Adipose tissue matures into white adipose tissue after about 2 to 3 months, which is significantly longer than the time it takes for muscles to develop. However, fat tissue must be simulated in order for the entire tissue to be simulated. Not only does it add flavour to meat, but it softens it as well. As a result of not having enough fat, the proof-of-concept hamburger was dry to the taste, according to the small group of tasters (Post & Hocquette, 2017). Taste, texture, or mouth-feel is influenced by several aspects of meat, including its tissue composition, its microscopic and macroscopic tissue architecture, and ability to retain moisture and fat after

cooking. In order to form small pieces of muscle, like in hamburgers or other type of processed meat, muscle cells and fibres must self-assemble in the previously mentioned matrix or scaffold. The arrangement and distribution of these self-assembled mature muscle fibres appear very similar to those of muscle fibres in vivo (Christov et al., 2007). As such, secondary structure in large bundles and ultimately a full-thickness muscle cannot be formed by self-assembly alone. It is important to note that such a tissue which is theoretically a steak in principle, would require additional technology to provide the macroscopic architecture of the tissue. Furthermore, the tissue would need to carry oxygen and nutrients into the core through perfusable channels. Although the technologies to make those structures already exist, they do not but have not been applied to meat and will require significant development (Vianney & Spitsbergen, 2011). The tissue-engineered muscle fibres possess cross-striations, strong evidence that actin and myosin complexes have formed on sarcomeres. Additional cells may be needed for full meat mimicry (Post & Hocquette, 2017).

Conclusion

In the coming years, the possibility of increase in total dietary intake of meat (both plant-based and conventional) is highly likely due to two reasons: (1) cell-cultured foods derived from livestock and poultry provide alternatives to conventional poultry and livestock production, which some find animal welfare and environmental-friendly, and (2) an increasingly prosperous population in some sub-sectors may exceed conventional production of animal-based food. Sustainable and novel sources of proteins are being explored and used in order to meet the ever-growing demand of high value protein foods like single-cell proteins. The products of different methodologies are different in attributes from one another, like the bottom-up strategy creates individual structural components from individual molecules or components including protein; however, the top-down approach makes anisotropic, fibrous materials predominantly from protein blends. 3D printing is one of the novel techniques, which has the ability to create meat analogue products as per the individual needs. It can be customized as per the customer requirements and characteristics of raw materials. Top-down strategy is more robust, scalable, and has better resource efficiency from a structural point of view, while bottom-up strategy mimics meat the closest. The different methods affect the quality of the meat analogue products in certain ways like structure, taste, and colour. In-depth research is needed in order to overcome those shortcomings. This in turn will help in creating structured foods that will mimic animal meat in all aspects of quality, and this will

help in tremendous growth of the meat analogue industry in the upcoming years.

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Data Availability No data are associated with the present work.

Declarations

Conflict of Interest The authors declare no competing interests.

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