



Plant-based meat analogs: A review with reference to formulation and gastrointestinal fate

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ABSTRACT

The discussion about the development and consumption of plant-based meat alternatives has been raised since numerous decades and has become the topic of prime concern these days. Recently, the market of plant-based meat alternatives has enormously expanded. With the aim of investigating the present scenario of research on meat analogs and defining the future research areas, reasons for shifting the trends towards consumption of meat analogs due to several health and environmental issues, potential sources and technologies needed for the development of meat analogs, physicochemical properties of meat analogs, functionality of ingredients used for manufacturing plant-based meat analogs, gastrointestinal fate of meat analogs and resulting consumer acceptability are summarized in this review. Studies have revealed that various health and environmental concerns are associated with the meat production which is the key driving force for the development of meat analogs. Recently, modern structuring techniques of plant-based meat alternatives have improved their functionality, however, a need exists to focus on improving the functionality, sensory characteristics, safety, and selection of suitable ingredients for the production of meat analogs. Additionally, the consumers' acceptability towards meat analogs is quite unsatisfactory which needs to be improved through proper research and creating awareness. Moreover, the gastrointestinal fate of the plant-based meat analogs needs further investigation in order to have a better understanding regarding the nutrient bioavailability of these products. The present review will be helpful in highlighting the current situation regarding the fate of meat analogs and opening new horizons of research in this domain.

1. Introduction

Consumption of plant-based meat alternatives seems to encounter various challenges despite the health and environmental concerns related to the meat production (Niva et al., 2017). Among these challenges, consumer's unwillingness to the dietary changes because of sensory and nutritional appeal of the meat-based products, and easy access to the meat products are of prime concern (Szejda et al., 2020; Corrin and Papadopoulos, 2017). However, plant-based meat analogs have gained much significance due to their established health claims and functionality. In addition to provide the similar nutritional value, the development of meat analogs is also focused on the modifications in the physical properties of meat analogs to improve the sustainability of these products through proper sensory attributes (Kyriakopoulou et al., 2019). Respective plant-based products which are available in the market include chunks, strips, patties, sausages, chicken-like blocks, nuggets, ground beef-like products and steaks etc.

Advancements in the processing techniques have resulted in the development of meat analogs with improved nutritional quality and physicochemical attributes (Kumar et al., 2017). Moreover, the market value of plant-based meat products has been expanded due to increased consumer acceptability. Studies have reported the projected growth of 8.6% in the market of meat analogs by 2025 with a total market share of USD 21 billion (Bohrer, 2019). According to the market research and competitive intelligence provider Fact.MR, the sale of meat alternatives has been significantly increased up to 38% from 2017 to 2021 (Fact.MR, 2022). Furthermore, the sensory, physicochemical, and functional characteristics of meat analogs are designed in a way to mimic the meat-based products by modifying their appearance, texture, mouthfeel, flavor, digestibility and bioavailability of the nutrients (He et al., 2020).

The functionality of various ingredients used in the formulation of plant-based meat alternatives i.e., proteins, water, lipids, carbohydrates, flavor substances, binding agents, and coloring ingredients, plays an important role in describing the properties of these products

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(Kyriakopoulou et al., 2021). For example, proteins from cereals, legumes and other sources are important to determine the nutritional and structural characteristics of meat analogs (Zhang et al., 2021). High-protein oilseeds such as sunflower and rapeseed are considered as the important components of meat analogs (Kyriakopoulou et al., 2021; Khazaei et al., 2019). The nutritional profile of plant-based meat analogs is improved by incorporating the proteins with balanced amino acid profile and lipids with low saturated fatty acid contents (Kyriakopoulou et al., 2021). Additionally, dietary fiber also plays a significant role in improving the textural properties and fluid-binding capacity of meat analogs (Bakhsh et al., 2021a). Likewise, the processing operations used in the manufacturing of plant-based meat alternatives such as mixing, shearing, extrusion, spinning etc., are also vital to obtain the desired functionality of meat analogs (Sha and Xiong, 2020).

At present, the understanding and knowledge about the physico-chemical properties of meat analogs, role of various ingredients to improve the functionality of plant-based meat alternatives and the gastrointestinal behavior of these products is quite limited. Therefore, this overview provides a comprehensive compilation of various studies describing the need of developing meat analogs, health concerns related to the consumption of meat products, formulation techniques of meat analogs, role of various ingredients in determining the functionality of meat alternatives and consumer acceptability of meat analogs. Additionally, this review article gives an overview about the gastrointestinal fate of meat analogs which will aid in providing the way forward for developing novel plant-based meat alternatives with improved nutritional quality and functional properties.

2. Health concerns and attitudes related to meat-based products

Traditionally, meat is a crucial part of human diet and evolution (Williams and Hill, 2017). Meat proteins have high biological value along with the significant quantities of important minerals (free iron, heme-iron, and zinc), and vitamins (B complex) resulting in significantly improved nutritional value of meat-based products. However different factors i.e., feed, breed, age, sex, species of animal, and the cut of the red meat affect the fat content of meat. Different meat processing methods such as barbecuing, grilling, or pan-frying (high-temperature cooking) result in the highest production of heterocyclic aromatic amines, N-nitroso-compounds (NOC), polycyclic aromatic hydrocarbons (PAH), and suspected carcinogens. Smoking and curing can be responsible to form NOC and PAH. While cooking may end in the production of suspected or known carcinogens counting HAA and PAH along with improving the palatability and digestibility of meat (Bouvard et al., 2015).

Meat comprised of cholesterol and a high content of saturated fatty acids compared to polyunsaturated fatty acids (PUFAs) that are associated with numerous chronic diseases which have caused proportions of an epidemic. Therefore, excessive consumption of meat products can't be commended from a health perspective (Kumar, 2016). High red meat diets especially processed meat are the well-known causes of several diseases such as cancers, obesity, cardiovascular disease, and type 2 diabetes.

The development of colorectal cancer has been linked with excessive consumption of red and processed meats (Hu et al., 2019). Also, there's a concern about other cancers like prostate cancer, pancreatic cancer, and breast cancer (Thavamani et al., 2020). The World Health Organization's International Agency for Research on Cancer (IARC), after an inclusive review of epidemiologic evidence, has classified processed meats (i.e., sausages, bacon, and hot dogs) as carcinogenic for colorectal cancer as well as unprocessed red meats (i.e., pork and beef) as "probably carcinogenic" to humans (Bouvard et al., 2015). Studies reported that an increased risk of diabetes in humans is associated with the consumption of processed meats. In diets, 50 g of additional processed meat is linked with the increase of fasting glucose in consumers. On the other hand, regardless of genetic risk scores, 100 g of additional

unprocessed meat can increase the concentration of insulin and the fasting glucose (Thavamani et al., 2020). In 2020, Zhong and his colleague conducted a cohort study of 29,682 US adults to find the association between meats (processed, red, poultry, and fish) intake and cardiovascular disease (CVD), and all-cause mortality. They found that, except for fish, consumption of unprocessed red meat, poultry, or processed meat showed a significant association with incident CVD. Also, unprocessed red meat or processed meat showed an association with all-cause mortality (Zhong et al., 2020).

Moreover, various adverse health concerns such as chances of zoonosis (like bovine spongiform encephalopathy, enteric bacterial infections, and viral infections), excessive use of hormones and veterinary antibiotics in animal farming can be associated with meat consumption (Ismail et al., 2020; van der Weele et al., 2019). The sustainable digestible food culture for human consumption encounters a change when unprecedented change occurs in livestock diet (Röös et al., 2017). Therefore, the focus of humans has shifted to plant-based meat from animal-based meat to choose positive health, animal welfare, and sustainability of the environment (Van Vliet et al., 2021). As aforementioned, the growth and demand for plant-based protein are significantly increasing globally, to replace the traditional dairy and meat products with new products, due to the concerns related to the consumption of animal-based products and their unfavorable effects on health and the environment (Tso and Forde, 2021).

Consumers are choosing more plant-based foods in replace meat, dairy, and eggs or declaring to be "flexitarian" to improve health or benefit the environment or both (Tso and Forde, 2021). Health-conscious consumers are more concerned in the ingredients, nutritional composition and health impacts of meat analogs. Findings of an investigation revealed that the meat substitutes have lower energy value, total fat contents, saturated fat and cholesterol levels as compared to the meat products which lowers the risk of obesity and cardiovascular diseases. Additionally, these products have high dietary fiber which is also helpful in improving the gastrointestinal health. Moreover, some meat analogs have also been fortified with several micronutrients such as vitamin B12, iron, zinc etc. Which further improves their health promoting functionality (Curtain and Grafenauer, 2019).

3. Meat analogs: from niche to mainstream

Since ancient times traditional products such as seitan, tofu, and tempeh have introduced the meat alternatives as a protein source concept. From 206 BC to 220 AD, the Han dynasty developed a standard meat analog "tofu" in China; that been consumed significantly throughout 618–907 AD (Tang dynasty). Later Tang or early Song dynasty, the consumption of tofu was spread to Japan (He et al., 2020). Later on, John Harvey Kellogg developed nut and cereal-based products (i.e., Nuttose and Protose) to foster good health in humans in the early twentieth century (Bakhsh et al., 2021a).

Furthermore, extruded wheat gluten, soy protein concentrates, or defatted soy meal helped to evolve the concept of dry texturized vegetable protein along with traditional Asian products (Lawrence and King, 2019). Significant developments were observed in textured proteins, plant protein isolates, and concentrates when noticeable signs of progress were observed in the packaging and production industries ensuing the second world war. Then, several industrialized nations were increasingly consuming meat through strengthened animal farming and expansion of agriculture. Thus, these developments reinforced the production of soy-based meat alternatives. In 1980, Tofurky and other similar products were developed to target the vegetarian demographic niche (Caputo et al., 2022).

Nowadays, meat substitutes are encountering a great expansion where in 2021 the estimated market value was USD 1.9 billion and now estimated to reach USD 4.0 billion by 2027, with a 13.5% recording compound annual growth rate (CAGR) (Markets, 2021). The top producers of plant-based meat analogs include Beyond Meat, Naturli'

Foods, Sainsbury's, Woolworths, Lightlife Foods, Vivera, Garden of Eatin', Nestlé, and Hain Celestia (Mintel, 2020). The trends “better for the planet” and “better for you” are associated with meat substitutes that are the chief drivers to increase the growth of market. These substitutes are gaining popularity as healthier protein sources in replacing meat consumption (Curtain and Grafenauer, 2019). Many studies have reported that high meat consumption is linked with different health issues (Bronzato and Durante, 2017), whereas meat substitutes are plant-based protein sources that are cholesterol-free and contain low saturated fats and health-beneficial essential amino acids (Guo et al., 2020).

The production of animal protein creates an environmental burden on the consumption of resources and global warming as well as animal welfare rights that has made the ethical consumer switch towards plant-based products (Hartmann and Siegrist, 2020; Siegrist and Hartmann, 2019). Plants (e.g., legumes) have positive impact on conserving biodiversity, agriculture, and preservation of soil fertility as underlined by the assessments of environmental research and life cycle (Boukid, 2021). Flexitarians, vegetarian, and vegans are the main rising health and ethical conscious niches that are prominently contributing to shape the meat analog markets; consisting of 78.1% vegan, 32% vegetarian, 37.1% high/added protein and 31.3% gluten free (Mintel, 2020).

The nutritional profile of plant-based meat analogs gets substantial attention of health-conscious consumers, while label readers additionally pay attention on the haleness of ingredients of meat substitutes (Boukid, 2021). Clean and natural label ingredients help the formulation in improving nutritional qualities of plant-based meat analogs. Brands utilize several genetically modified (GMO) and/or heavily processed ingredients in meat analogs to mimic the taste, appearance, and texture meat (Chuck et al., 2016). In 2020, Mintel reported that world has faced drastic shift from GMO to GMO-free products with a launch of 39% GMO-free products in the recent years. Nowadays, consumers associate healthiness of food to the chemical free, recognizable and natural foods that is rising the focus on naturalness of the ingredients. For instance, 62% launched products have been claimed natural since 2015 (Mintel, 2020). Therefore, application of clean label ingredients can further strengthen the meat analog position in the market for all consumers focusing healthier food options including vegetarians and vegans (Boukid, 2021).

4. Formulation of plant-based alternatives to meat

Meat is a crucial part of the human diet, but vegetarians consume meat alternatives to meet their protein requirement (Singh et al., 2021). The nutritional requirements of humans always alter according to the food availability, location, and climatic conditions. However, plant-based meat analogs are replacing meat protein sources due to health, environmental and cultural reasons that are gaining traction in food markets around the globe. The major constituents of meat analogs formulation include soy, legumes, cereals, mycoprotein, coloring agents, flavor, and other ingredients (Michel et al., 2021). A general illustration of formulating the plant-based meat analogs is given in Fig. 1.

4.1. Soybean

Soybean is an easily available and extensively used ingredient in meat analogs worldwide. It has been renowned for its excellent nutritional and functional attributes; abundant in carbohydrates, fat, fiber, vitamins, micro- and macro-nutrients (Bakhsh et al., 2021a). Historically, it has been used in various Buddhist food recipes such as they used soy protein isolates, soy protein concentrates, and soy deflated flour to develop fabricated soy products (Sha and Xiong, 2020). Kumar and his colleagues substantially reported that due to proportionate nutrients content of soybean being used to replace red meat (Kumar et al., 2017). Soybean protein has designated the maximum possible score of 1.0 for being identical to the animal protein on the scale of Protein Digestibility

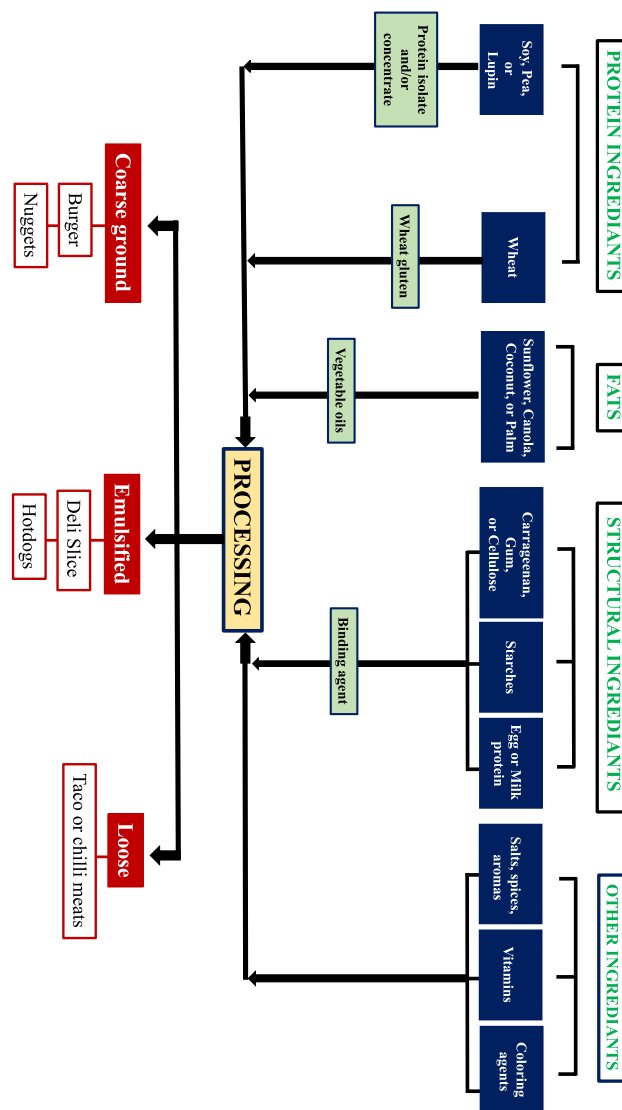


Fig. 1. Formulating the plant-based meat analogs.

Corrected Amino Acid Score (Bakhsh et al., 2021a). Its proteins have been used for individuals with cardiovascular disease as they lower blood cholesterol (Kumar et al., 2017). A mixture of non-textured and textured soy proteins is used to manufacture the meat analogs (Mäkinen et al., 2017). Soy proteins have been used to manufacture different products such as chicken-style nuggets and breasts, meat-free sausages, and sliced cooked meats similar products (Bakhsh et al., 2021a). Defatted soy flour is utilized to obtain textured-vegetable protein by detaching soluble carbohydrates and texturing the filtrate by spinning or extrusion (He et al., 2020). The textured soy protein concentrates mimic the fibers of meat muscle i.e., fibers of turkey or chicken breast meat (Yao et al., 2006); gives meat analog fibrous characteristics like mouthfeel, chewiness, and hardness (Chiang et al., 2019).

4.2. Legumes

Globally, primary crop production has 27% of the *Leguminosae* family comprising of pulses and beans. It stands second to cereal grasses in importance. Legumes-based diet is an excellent source of protein (Riascos et al., 2010). Different characteristics like foam stabilization and gel formation of the pulse crop (such as chickpea, lupine, lentil) and

bean crop have been evaluated and established that pea protein is the most suitable to be used in the meat analog formulation (Osen et al., 2014). Pea protein-based meat analog, having a similar fibrous texture to fish and chicken meat, has been effectively formulated by researchers previously; that contained 90% protein from pea protein isolates, 80% protein from gluten, and high moisture starch to improve the hydration properties of the product. Legumes are abundant in protein, starch, fiber, and essential amino acids (like arginine, lysine, glutamic acid, leucine, and aspartic acid), therefore, an essential part of human nutrition (Bakhsh et al., 2021b). Unprocessed legume products offer more protein digestibility than proteins derived from animal sources (Huang et al., 2018). Legumes-based meat is reporting a remarkable increase based on its functional properties, quality, texture, and economic importance (Malav et al., 2015).

4.3. Cereal proteins

Cereal crops are a central part of the primary food crop production. The food processing industry substantially relies on the products obtained from cereal grains (Bakhsh et al., 2021a). Cereal grains are inferior to legume grains in protein content. The primary source of cereal proteins that are fibrous and proteinaceous include wheat, rice, barley, and oats (Shewry, 2009). Wheat gluten is economical and has viscoelastic properties that assist the wheat protein to mimic meat texture through treatment and extrusion process. Therefore, wheat gluten is particularly included in the formulation of meat analogs amongst all other cereals (Kyriakopoulou et al., 2019). Ingredients of textured vegetable protein can be obtained from food products composed of wheat gluten. These ingredients can be used as meat analog products and meat extenders. For instance, gluten from in-grounded meat patties possible to applied as a binder and an extended to develop rearranged items. Besides this, various types of meat substitutes can be produced by converting hydrated gluten into fibers through an extrusion and texturizing process (Malav et al., 2015).

4.4. Coloring ingredients

Color and color variations significantly influence the quality of meat substitutes. Hence, coloring ingredients are crucial part of meat substitute formulation. Consumers prefer the use of heat-stable coloring ingredients like pigments of turmin, carotene, and cumin (Vrljic et al., 2018). The red meat color in meat substitutes is mimicked by adding leghemoglobin, annatto extracts, beet juice extract, or lycopene, while chicken color is mimicked by adding titanium dioxide (Beyond Meat, 2020; Schreuders et al., 2019). However, stability of coloring ingredients is ensured by adding polyphenols and ascorbic acid rich juices or direct addition of ascorbic acid; act as preservative and antimicrobial agents (Schreuders et al., 2019). Rolan et al. stated that development of meat analog color depends upon the color formation through different combinations of reducing sugars and heat-labile colorants (Rolan et al., 2008). Plant-based meat analogs are colored either by applying coloring solutions, mixing coloring ingredients with protein material before extrusion process or injecting into the barrel of extruder (Orcutt et al., 2008).

4.5. Flavoring ingredients

The average consumer criteria for the acceptance of meat substitute are a significant taste and flavor (Kyriakopoulou et al., 2019). The aromatic profile of meat substitute is compensated by using different flavoring ingredients including spices, herbs, savory yeast extract, paprika and sugar (Boukid, 2021). Sulfur and sugar (containing amino acids) play integral role in the development of meat flavor. Furthermore, flavoring ingredients such as or hydrolyzed vegetable protein or monosodium glutamate can be replaced with mushroom concentrate for flavor enhancement and flavor formulation (Bakhsh et al., 2021a, b).

5. Physicochemical and functional characteristics of plant-based meat analogs

Meat analogs realistically imitate the structure and functionality of the meat. Consumers require specific characteristics of the product such as physicochemical, textural and sensorial attributes before buying (Dekkers et al., 2018). Therefore, meat analog must resemble the meat, to attract those consumers who mostly consume meat, as to appearance, texture, smell, and taste (De Angelis et al., 2020). The principal constituent of meat analog formulation is texturized vegetable proteins which mimic the fibrillar structure of meat muscle (Caporgno et al., 2020).

Table 1 summarizes the types, dosage and functionality of plant proteins which affect the physicochemical and functional attributes of plant-based meat analogs. Several studies have explored the role of different plant proteins in modifying the functional attributes of meat analogs. For instance, Lee et al. (2022) compared the effectiveness of soy protein isolate with rice protein isolate to formulate the meat analogs and concluded that the combination of both protein isolates improved the water absorption capacity, porosity, and specific mechanical energy of the meat analogs. In another investigation, Chiang et al. (2021) reported that using 100% wheat gluten to develop meat analogs resulted in the poor chewiness of the product however, adding soy protein isolate improved the hardness and chewability of the final product. Similarly, Bakhsh et al. (2021b) prepared meat analogs by using texturized soy protein isolates (T-SPI) and texturized vegetable protein isolates (TVP) with varied concentration of methylcellulose (1.5%, 3%, 4%) and recorded good textural profile and crude fiber percentages in the resultant products. In another investigation, different plant-based meat analogs were prepared from soy protein isolate, pea protein isolate and pea protein dry-fractionated isolate by incorporating the oat proteins (30%) and stated that analogs prepared from pea-based extrudates showed good oil absorption capacity and sensory profile, whereas lower hardness was recorded in the products prepared with pea protein dry-fractionated isolate (De Angelis et al., 2020). Likewise, plant proteins are also reported to develop the restructured products with good texture profile and fibrous structure along with similar integrity index, nitrogen solubility, cutting strength, and chewiness to chicken sample (Chiang et al., 2019; Samard and Ryu, 2019). These findings provide a strong evidence that plant proteins play a very important role in modulating the physico-chemical characteristics of meat analogs. However, more comprehensive studies are required to further explore their potential to develop improved plant-based meat analogs.

Protein aggregates are converted to the fibrous texture through texturization process. Vegetable-based proteins could be restructured to develop fibrous textures resembling to the structures of meat tissues through extruders (Sarmad and Ryu, 2019). Mostly, high-moisture extrusion cooking technology is employed to develop muscle meat like big pieces of texturized protein (De Angelis et al., 2020). Furthermore, extrusion cooking enhances the nutritional value of pulses by reducing the available antinutritional factors (Pasqualone et al., 2020). Certainly, legumes have less acceptability and nutritional value due to the presence of digestive enzymes inhibitors, tannins and phytic acids in them (Samtiya et al., 2020). Pasqualone and his colleagues outlined that combination of mechanical shear and thermal treatment of the screw speed in extraction process can reduce heat-unstable compounds such as tannins, phytic acid, and trypsin inhibitor (Pasqualone et al., 2020); increase protein content, enhance the starch digestibility, and develop certain important edible products for both developed and developing regions around the world (De Angelis et al., 2020).

Meat and meat products' texture, flavor, mouthfeel, and juiciness are greatly contributed by animal fats (Sha and Xiong, 2020); also contribute to the nutritional value, sensorial and textural properties of meat and its substitutes. Besides this, fat carries fat-soluble vitamins and flavour. Coconut and cocoa beans (tropical fruits) extracted solid fats are mixed with unsaturated fatty acids high canola and sunflower oil

Table 1
Physicochemical and functional parameters of plant-based meat analogs.

| Key ingredients | Concentration | Parameters analyzed | Functionality/major findings | References |
|--|---|---|--|--------------------------|
| - Soy protein isolate (ISP) | IRP at 25, 50, 75, and 100% | - Physicochemical properties | - IRP replacement showed a decreased water absorption capacity, porosity, and specific mechanical energy of the meat analogs | Lee et al. (2022) |
| - Rice protein isolate (IRP) | | - Compared to commercial TVP product | - Compared to commercial, IRP and ISP combinations resulted in better nutritional quality - IRP can partially replace ISP in the manufacture of meat analogs - IRP can be used to partially replace ISP to manufacture meat analogs | |
| - Wheat gluten (WG) | WG at 40, 60, 80, and 100% | - Physicochemical properties | - Lowest hardness and chewiness were reported in 100% WG meat analogs | Chiang et al. (2021) |
| - ISP | | - Textural characteristics - Structural parameters | - Ultrastructure was insignificantly impacted by the WG proportional concentration - Compared to steamed chicken, meat analogs showed less lysine contents even after increasing ISP concentrations to improve lysine contents - Meat analogs showed unique compact ultrastructure under scanning electron microscopy (SEM) compared to controls | |
| - Textured soy protein isolate (T-SPI) | MC at 1.5, 3, and 4% | - Physicochemical properties | - TVP and T-SPI reported higher pH values | Bakhsh et al. (2021b) |
| - Textured vegetable protein (TVP) | | - Textural characteristics | - TVP had higher crude fiber | |
| - Methylcellulose Concentration (MC) | | - Sensory attributes - Compared to control (beef patty) | - Control reported high texture profile analysis (TPA) including hardness, gumminess, and chewiness, and Warner-Bratzler shear force | |
| - Soy protein isolated (SIs) | PIs_OP: (70:30 w/w); PDF_PIs_OP: (35:35:30 w/w/w); Sis_OP: (70:30 w/w); PDF_OP: (70:30 w/w) | - Physicochemical properties | - High oil absorption capacity resulted in pea-based extrudates | De Angelis et al. (2020) |
| - Pea protein isolated (PIs) | | - Sensory attributes | - More neutral sensory characteristics reported in protein isolates based extrudes - Lower hardness was observed in PDF-based meat analogs than others | |
| - Pea protein dry-fractionated (PDF) | | | | |
| - Oat protein (OP) | | | | |
| - Wheat gluten (WG) | WG at 0, 10, 20 and 30% | - Physicochemical properties | - The highest texturization degree, fibrous structure, hardness and chewiness was found in 30%WG | Chiang et al. (2019) |
| - Soy protein concentrate (SPC) | | - Textural characteristics | - Fibrous structures interconnected with much smaller fibers were exhibited in 30%WG - Hydrogen bonds were found to be linked with a large portion of protein in meat analog | |
| - Wheat gluten (WG) | WG at 60% | - Physicochemical properties | - TVP showed similar integrity index, nitrogen solubility, cutting strength, and chewiness to chicken sample | Samard and Ryu, (2019) |
| - Isolated soy protein (ISP) | | - Textural characteristics - Structural parameters - Compared to lean meats | - TVP had significantly higher water absorption capacity - TVP exhibited different color and amino acids - A fibrous structure with non-uniform air cells shown in TVP compared to all other meat samples | |
| - Vital wheat gluten (WG) | - Formula 1: ISP at 90% and CS at 10% | - Physicochemical properties | - 40%WG showed lowest texture stability, integrity index, and higher nitrogen solubility index along with a meat-like distinctive dense and fibrous structure | Samard et al. (2019) |
| - Corn starch (CS) | - Formula 2: ISP at 50%, WG at 40%, and CS at 10% | | | |
| - Isolated soy protein (ISP) | | | | |

(vegetable oils) to develop animal fat like texture and mouthfeel (McClements and Grossmann, 2021; Kyriakopoulou et al., 2021). Different origin oils such as avocado and sesame oil are utilized to improve the taste and fatty acid profile of products. Blends of oils (saturated and unsaturated) are beaten into small fat globules that are used to mimic the marbling appearance in plant-based “sausages” and “burgers” like regular ground animal sausage patties (Dreher et al., 2021). Noteworthy, animal fat naturally contains aldehydes, alcohols, sulfur compounds, and hydrocarbons. While plant fats lack fat-soluble flavor contributing volatiles (Arshad et al., 2018). Still, plant fats have the status of healthier fats due to cholesterol free and high unsaturated fatty acids content from a nutritional perspective (Fu et al., 2021).

Color plays an essential role in the acceptability of final food product. Commonly, the red color of fresh meat (raw meat) turns to brown on cooking. Hence, plant-based meat analogs require to mimic the initial and final color (during and after cooking) of meat. Originally, gluten and soy proteins possess beige or yellow color. Different coloring ingredients are used in meat analogs. Therefore, successful application of coloring

ingredients in meat analogs require to meet pH sensitivity and thermal stability. Since thermally unstable coloring ingredients undergo degradation and result unacceptable color appearance in meat analogs upon cooking. Therefore, pH range of coloring ingredient must match to maintain the optimum coloring effect in meat analogs (He et al., 2020). Typically, malt extracts and caramel color are heat stable and bring brown color appearance in the final meat analog (Kyriakopoulou et al., 2019b).

6. Nutritional composition of plant-based meat substitutes

While developing the plant-based meat substitutes, the priority is given to design a product with same organoleptic characteristics as meat and meeting the nutritional specifications of the targeted meat product (Kyriakopoulou et al., 2019). Among the macronutrients, the prime focus is given to the protein percentage. Additionally, to maintain the similar functional and nutritional properties as of the meat products, moisture and lipid levels of the meat analogs are also adjusted. Hence, it

can be stated that the meat analogs are quite similar to the conventional meat products in terms of nutritional value particularly the macro-nutrients.

Currently, beef burger simulation products are considered as the most popular meat substitutes. Several food service and retail products fall in this category. While characterizing the nutritional value of beef products, McDonald's beef patty and cooked ground beef (lean = 93%, fat = 7%) are considered as the standard meat products. According to the USDA Nutrient Database (USDA, U.S. Department of Agriculture, 2019), ground beef has a caloric value of 182 kcal/100 g with 25.56% total protein content, 8.01% total fat, 3.29% saturated fat, 0.084% cholesterol, 0.072% sodium and 0.0028% iron with no carbohydrates and dietary fiber. Additionally, the nutrient specifications of McDonald's beef patty include 23.33% protein content, 20% total fat percentage, 8.33% saturated fat, 0.083% cholesterol, 0.4% sodium and 0.003% iron with no carbohydrates and dietary fiber and having a total caloric value of 266.67 kcal/100 g.

Anderson et al. (2009) evaluated meat substitutes that simulate beef burger products and found similar nutritional profile as of the meat i.e., McDonald's beef patty. The two products namely, Beyond burger and Impossible burger, were found similar to the McDonald's beef patty in terms of total fat contents i.e., 15.93% and 12.39% as compared to 20% in meat, and saturated fat contents i.e., 5.31% and 7.08% as compared to 8.33% in meat. In another formulation, two different meatball products were compared with a meat analog (Gardein meatless meat balls). The nutritional analysis revealed that the meat analog had a similar protein content of 15.56%. However, a lower energy value (166.67 kcal/100 g versus 300.00 kcal/100 g), fat content (7.78 g/100 g versus 16.47 g/100 g), saturated fat (0.56 g/100 g versus 5.88 g/100 g) and cholesterol level (0.00 mg/100 g versus 47.06 mg/100 g) was recorded.

7. Digestibility and gastrointestinal fate of plant-based meat analogs

Plant-based meat analogs are designed to improve the nutritional functionality of the product. Generally, the plant-based meat analogs contain high protein with essential amino acids and low-fat content which makes these products good for human health (Kyriakopoulou et al., 2021). Additionally, the textural and fluid/water holding capacity of the meat analogs are improved by adding dietary fiber to the product (Bakhsh et al., 2021a). Despite considerable nutritional characteristics, the behavior of these nutrients in the gastrointestinal tract is still questionable (Lee et al., 2020). Therefore, the proper understanding about the mechanism of gastrointestinal fate of the plant-based meat analogs is very important which can be useful to get a better knowhow about the digestibility and bioavailability of meat analogs (Ogawa et al., 2018).

The compositional and structural properties of plant-based meat analogs are quite different from the meat-based products which describes the difference in the gastrointestinal fate of meat analogs. Moreover, addition of various coloring agents, flavoring substances and other food additives is responsible for variation in the digestion, absorption and assimilation of nutrients present in the meat analogs (Bakhsh et al., 2021a; De Marchi et al., 2021). Similarly, the processing steps of meat analogs are different from the processing of meat products which changes their gastrointestinal fate. In this perspective, Zhou et al. (2021) studied the properties of beef and beef analogs to compare their gastrointestinal fate with the help of *in vitro* digestion model (INFOGEST). The physicochemical and microstructural characteristics of meat and meat analog were compared to study the digestion rates of both products by using the model gut. The results of the investigation concluded that the addition of dietary fiber in the meat analog resulted in the slow digestion of lipids in the small intestine. This behavior was attributed to the increase in the viscosity of plant lipids in the gastrointestinal fluids. However, a rapid digestion of plant proteins was

observed as compared to the meat proteins in the stomach followed by a slow digestion in the small intestine due to different type and structure of the proteins and presence of dietary fiber in the meat analog.

In another investigation, the behavior of different plant protein isolates during the gastrointestinal digestion was explored by primarily focusing on the characterization of resistant factors to the digestion process and amino acid profiling. Purposely, proteins from four different sources i.e., grass pea, garden pea, lentil and soybean were studied for their degradation by using the harmonized Infogest *in vitro* digestion protocol. Results stated that soybean protein isolate showed highest insoluble nitrogen percentage after the completion of digestion process i.e., 12% primarily containing hydrophobic amino acids. It was also recorded that free amino acids comprised of 21–24% of the total nitrogen content and released during the intestinal digestion. Moreover, legume proteins were hydrolyzed to amino acids and peptides during the intestinal digestion however, showed resistant to the gastric digestion. The study concluded that legume protein isolates were the efficient source of essential amino acids. However, analyzing the proper gastrointestinal fate of plant proteins need to be properly investigated for better understanding of their digestibility behavior (Santos-Hernández et al., 2020).

It is generally claimed that several ingredients added in the formulation of meat analogs hinder or delay the digestion process. It is, therefore, suggested to keep the formulation of meat analogs simple by excluding the ingredients which obstruct the digestion process. Several anti-nutritional factors are responsible to limit the digestibility of plant proteins. The main phytochemicals in this perspective are tannins, phytates, lectins and trypsin inhibitors. Therefore, inactivation of these anti-nutritional factors is important to improve the digestibility of plant proteins. Studies have revealed that several processing techniques such as conventional cooking, extrusion, autoclaving, fermentation, microwave processing, freeze-drying and irradiation improve the quality of plant proteins (Sá et al., 2019), however, the impact of these processing techniques on the digestibility of plant proteins needs further exploration. Moreover, simulated gastrointestinal models should be used to study the digestion fate of nutrients present in the plant-based meat analogs. Likewise, *in vivo* trials should be conducted to assess the differences in the bioavailability and nutritional value of meat and meat analogs by studying their rates of absorption in the gastrointestinal tract.

8. Quality and safety attributes of plant-based meat alternatives

The scientists are now focusing on the research and development of the plant-based meat alternatives. Factors like human health, environmental sustainability and animal welfare effect the development of plant-based meat alternatives. Plant base meat alternatives were 1st time introduced to world by 1960s but in Asian civilization the raw idea of plant-based meat analogs was practiced centuries ago analogs. Texture, flavors, biological and chemical safety control and protein sources influence the production of plant-based meat analogs. To bring improvement in the overall product quality and development processes different techniques like sheer cell techniques and extrusion techniques are studied extensively. The acceptance of the meat alternatives depends on the consumer awareness about the meat alternatives, educating them about the health benefits of plant-based meat analogs with scientific references. Consumer acceptance can be improved by improving flavors and appearance. Quality of the final products can be improved by finding better protein sources and ensuring the better safety standards of the processing of plant-based meat analogs. (He et al., 2020).

Recently, the formulation and nutritional composition of 7 meat alternatives products were studied. It was found that each product contained 20-30 additives according to the ingredient list. The products that were analyzed were burgers, nuggets and nuggets (Bohrer, 2019). The presence of high amount salts and saturated fats actually make plant-based meat alternatives doubtful about the health and nutrition claims. Similarly, the production of toxicants and carcinogenic

substances like heterocyclic aromatic amines during the high temperature processing of protein foods. (Barzegar et al., 2019). These toxicants were detected during the high temperature processes like grilling, roasting, baking and frying (Jiang and Xiong, 2016; ur Rahman et al., 2014). Similarly, the plant based meat alternatives that high protein contents are subjected to the high temperature treatments like baking, frying and grilling, increase the susceptibility of such toxicants. Naturally occurring phenolic compounds act as inhibitors against the formation of toxicants thus improving safety of the plant based meat alternative products (He et al., 2020; Lu et al., 2018; Xiong, 2017; Wild et al., 2014; Hoek et al., 2013).

Normally, 10–14% of the patients of lactose intolerance can be allergic to the soymilk products (He et al., 2020; Sicherer, 2005). Maize zein, was used as major source of gluten free protein that contain properties of the meat alternatives which can ultimately help to prevent the allergic responses (He et al., 2020). Consumers are conscious regarding the effect of genetic modification of soybeans on the meat analogs produced from the soybeans. Genetic Modification technology has helped to improve the nutritional and physical characteristics, but people have doubts about the potential negative effects on the human health. The red color and flavor of meat is because of the presence heme, iron containing molecule, present in the muscles of the animals (He et al., 2020). In the plant-based meat products such as the Impossible Burger, heme is introduced with help of genetically engineered yeast by the addition of soy leghemoglobin gene to a strain of yeast, there promoting the growth of yeast through the process of fermentation and finally isolating the heme from it. Aroma, favor and cooking properties are enhanced by this heme (Jiang et al., 2008). Moreover, there is need of more laboratory evidence to establish the safety standards (He et al., 2020). The characteristics like texture, flavor, and moisture content similar to the meat are influenced by the microstructures of plant-based meat analogs (Espeleta and Mora, 2010). The major problem faced during the production of meat analogs is the absence of fibrous structure, tenderness and juicy mouth feel because the alternative products lack these both character due to the processing method of extrusion. However, optimization of processing technology and formulation for plant-based meat analogs is useful in the development of these characters in the products (He et al., 2020).

Advanced technology has ensured food safety and security in the world along with giving the freedom of choice to the customers as the product variety has increased with the improvement in technology (Grunert, 2005; Wilcock et al., 2004; Piggott and Marsh, 2004). Nowadays, drugs and pesticide residues are the crucial topic regarding food safety as they can be found in the food products of animal origin (Stephens et al., 2018). Another important concern regarding food safety is the usage of hormones and pesticides (Sha and Xiong, 2020) and this concern has influenced the livestock farming as the cattle are exposed to growth hormones, antibiotics and synthetic pesticides (IFOAM, 2005; Webster, 2002). Wide use of antibiotics is raising many health issues like disease epidemics such as swine influenza that's why consumers are more conscious about the drug administration on animals (Williams and Hammitt, 2000). Customers are more interested to consume the meat products that are drug free (Sha and Xiong, 2020; Williams and Hammitt, 2001). Cultured meat is regarded as the clean meat because none of the hormone or antibiotic is added in it as compared to the animal meat (Bryant and Barnett, 2018; Specht et al., 2018; Jeong et al., 2010). However, cultured meat is made from a laboratory (Hwang et al., 2020) but plant meat products are derived from the plant sources and are thought to be safe for human consumption (Sha and Xiong, 2020; Vanhonacker et al., 2013). People are cautious about the food due to the concerns of the application of technology during processing (Cox and Evans, 2008; Pusztai and Bardocz, 2006) because they are concerned about the possible effects of technology in the long run (Sha and Xiong, 2020).

The allergenicity of few proteins from plant sources is regarded as risk for the wellbeing of human beings like allergenicity of IgE-binding

G2 glycinin that is soy protein (Helm et al., 2000; McClain et al., 2014). The plant protein allergens are associated with three families of the proteins—namely, prolifins, storage proteins and pathogenesis-related proteins. These allergens elicit IgE-mediated immunological reactions resulting in several respiratory, gastrointestinal, skin-related and cardiovascular ailments (Verma et al., 2013; Hadi and Brightwell, 2021). Several studies have revealed that thermal processing, ultrasound treatment, high pressure processing etc. During the formulation of plant-based meat analogs can significantly mitigate the allergenicity of plant proteins (Verma et al., 2012; Cabanillas et al., 2018). However, limited data related to the allergenicity of the plant-based meat analogs is available therefore, this parameter needs to be extensively studied through further clinical investigations for exploring the real mechanisms and physiological effects in live hosts. Additionally, future studies should be directed to define the regulations related to the development, marketing and consumption of plant-based meat analogs.

Though the nutritional benefits of soy proteins are undeniable but inconsistent research results have led to the cancellation of its health claim by Food and Drug Administration (FDA) that were granted in 1999 (FDA, 2017). However, gluten intolerance and allergy are another potential risk factor in the products containing the wheat proteins (Miller, 2018). The effect of different potential handling and storage conditions like temperature and packaging after the processing of the products, on the flavor change and microbial safety of products must also be studied (Sha and Xiong, 2020).

Recently, due to the negative impacts of meat on economy, ecological and human health has fueled the controversies about the production and consumption of meat. Extensive use of water and land resources and greenhouse gas emissions relatively higher in the meat production as compared to the plant-based food production is threatening the environment. Similarly, meat production is also posing many health issues like increased risk of CVDs, foodborne infections, colorectal cancer. Research have shown that there is possibility of transfer of growth promoters and antibiotics that are taken in form of veterinary medicines, from animals to human beings thus risking the human health (Verain et al., 2015; Pathak et al., 2010; Mugerza et al., 2004). The practice of meat production has raised many problems regarding ethical issues and animal welfare due to the caging and slaughtering practices (Kolbe, 2018). For the scientists and policy makers, the consumption of meat is very important issue that needs to be addressed with care due to its sensitivity (Reisch et al., 2013). In the recent times, the consumption of meat has increased. The meat substitutes that are plant-based products and rich in proteins are introduced as alternatives that are thought to be more sustainable (Elzerman et al., 2015; van Mierlo et al., 2017). Meat alternatives can contain many plant-based constituents that have similar characteristics to meat like nutritional value and appearance such plant-based ingredients could be from rice, wheat, soy, mushrooms and pulses (Malav et al., 2015). So, consumers either vegetarian or non-vegetarian procure meat alternatives in order to avoid the ethical, health and environmental issues. However, the demand of meat alternatives is still very low (Elzerman et al., 2015; Kim et al., 2011). Many monitoring systems have been developed that are running online and helps to study the quality of lab meat and ensure food safety thus resulting in the minimum bacterial contamination in the processing. The quality standards of the cultured meat are improved by controlling the culture system and post processing conditions like nutrient content, composition and flavor (Zhang et al., 2020).

9. Consumer acceptance of plant-based meat analogs

With a growing population, the demand for meat is also increasing. Rearing of the animal for meat consumption utilizes a large number of resources and creates many ethical issues (Kumar et al., 2017). Because of the increasing awareness consumers are in a conflicted situation about the consumption of meat (Kumar et al., 2017). In this scenario people desire to consume meat but they don't want to be linked with the

moral and ethical issues (Buttlar and Walther, 2018). This conflicted situation gave scientists an idea to introduce alternative meat sources that promote the idea of animal welfare and provide customers with solutions for this conflict (Wilks et al., 2019; Specht et al., 2018). Plant based meat analogs are considered to have therapeutic benefits for human beings. The consumption of meat analogs that are supplemented with dietary fiber help in the improvement of gut health and prevents obesity and diabetes (He et al., 2020). As we know, the food industry revolves around the goals of consumer health and food safety. Consumption of soybean products is dated back to two thousand centuries in Asia while in western world consumption of soy products was adopted a few decades ago and now are the popular source of proteins for human beings (He et al., 2020). Moreover, there are studies which indicate the negative effects on the cognitive health of human beings (Hwang et al., 2020; Wild et al., 2014). Unawareness is an important barrier in consumer's food choice and normally are not interested in novel food products. (Verbeke, 2015).

The production of meat free products that are tasty and healthy, has been increased as people are considering meat to be harmful for the environment. (Kumar et al., 2017; Hoek et al., 2011a,b). Consumers are tending towards the vegetarian diet as they are concerned about the welfare of the animals (Janda and Trocchia, 2001). Innovation in the field of food technology is expanding the options for the sustainable meat alternative thus providing the proteins for the vegetarians as well as for the people who are not purely on the vegetarian diets. (Mohamed et al., 2017; Elzerman et al., 2015), that's why the scope of development of the meat alternative food product market is expected to increase in the near future (Kim et al., 2011). Acceptance of the novel product is vital for the success for the food product development. (Siegrist, 2008). The success and acceptability of the meat and meat substitutes depends on many factors that may include economic, personal and cultural issues. Consumers are reluctant to use the meat alternatives as they consider them to be unhealthy, taste less and they choose other sources of proteins like lentils to be a better source of proteins. (Hoek et al., 2011a,b). The cost of meat affects the rate of meat preference for the consumption significantly (Smart, 2004). Few consumers are confused in choosing such foods as their preferences are influenced by the texture, appearance, taste and juiciness (Resurreccion, 2004). On these preferences, consumers are divided into two groups; sensory vegetarians, who reject foods that resemble meat and mainstream vegetarians who show acceptability to the meat alternatives (Rivera and Shani, 2013). Consumers are not readily accepting the meat substitutes as this new food category is perceived to have more distinct quality as compared to the meat (Hoek et al., 2011a,b). In the past studies, it was found that the consumers consider meat as superior food or they are entitled to have, considering it to be more flavors and healthier than the alternatives of meat. (Elzerman et al., 2015; Hoek et al., 2011a,b). Yet, proper marketing and consumer awareness campaigns about the health and ecological benefits of meat and meat substitutes can help consumers in the decision-making process for the preference and consumption of meat and meat alternatives (Cheah et al., 2020; Vinnari et al., 2010). Pricing plays an important role in the success of the meat and meat alternatives among consumers. (Revoredo-Giha and Costa-Font, 2018; Wirth, 2015).

9.1. Uncertainty of the consumer behavior

Consumers have contradictory approaches towards the meat consumption as they want to eat meat but without affecting the animal wellbeing. (Macdiarmid et al., 2016). Studies have shown that the consumption of meat has pros and cons on the health of consumer (Wolk, 2017; Henschion et al., 2014). In the past, research have shown that meat alternatives have positive impact on the sustainable agriculture as these are more sustainable as compared to meat products and have better impact on the environment resources (Sabate and Soret, 2014). Additionally, meat alternatives are free from antibiotics and hormones as mass meat production is not the goal (Bryant and Barnett,

2019). Broad (2020) found that the consumer is conscious about the consumption of meat alternatives as this class of food products is considered as a novel food.

However, the health benefits and functionality of these products are not fully explored on the scientific grounds which creates the hurdles in the consumer acceptance of these novel products (Circus and Robison, 2019). In another study it was found that the people are scared of the possible hazards of this technology though the United States Department of Agriculture (USDA) and the Food and Drug Administration (FDA) have permitted the consumption of these alternative meats (Verbeke et al., 2015). Similarly, Consumers have less awareness regarding the functionality of meat analogs, so they experience food neophobia. (Hwang et al., 2020; Gómez-Luciano et al., 2019). Consumers perceive the meat analogs as unusual and unhealthy products with a bad taste. However, a class of consumers has a positive attitude towards the consumption of meat alternatives because of the good taste and sustainability (Tosun et al., 2021). Hence, it is required to explore the evidence-based functional and health characteristics of these products to enhance the consumers' confidence regarding the consumption of meat products.

9.2. Factors influencing consumer acceptability of meat analogs

Trend towards adopting the flexitarian and plant-based eating patterns has been increasing globally (Estell et al., 2021). The key motivators behind this shift can be categorized into as traditional drivers e.g., convenience, cost and taste; and emerging drivers such as safety, health, environment and animal welfare (Curtain and Grafenauer, 2019). In this perspective, Apostolidis and McLeay (2016) concluded that the consumers' acceptability towards meat or plant-based meat substitutes depends on several factors such as type of meat, total fat content, price, environmental impact and brand etc. Which suggests the complexity of consumers' choice (Curtain and Grafenauer, 2019). The additional factors influencing the people's choice towards purchasing the plant-based meat analogs include age, gender, income, education level and geography (Neff et al., 2018). In term of age, the preference of millennials towards meat analogs was mainly govern by the environmental concerns and convenience whereas, the acceptability of older people (45–59) was mainly due to the familiarity and taste of the product (Neff et al., 2018; Bryant et al., 2019). Moreover, the reduced consumption of meat products was more common in the low-income populations as compared to the high-income populations (Neff et al., 2018). In terms of gender, shifting towards consuming the plant-based meat alternatives was higher in females as compared to the males (Rozin et al., 2012). Additionally, the young females (age 18 to 34) with higher education levels were more concerned about the environmental concerns of meat products, whereas the prime reason of shifting to the meat analogs among females over 55 years of age was the health-related issues of meat consumption (Bryant et al., 2019).

Hence, these investigations provide a strong evidence that the dietary behavior and trend towards shifting to the meat substitutes is highly associated with the consumers' motivation for purchasing and consuming the plant-based meat analogs. These studies revealed that the trend of consuming the meat alternatives among the conventional eaters is primarily due to the emotional connection and taste profile (Milford et al., 2019), whereas the prime factor for consuming the plant-based meat substitutes among vegans and vegetarians is the ethical concern. These studies also conclude that flexitarians are generally motivated towards consuming the plant-based meat analogs due to health, ethical and environmental concerns (Graça et al., 2015; Neff et al., 2018).

10. Conclusion and future outlook

The topic of plant-based meat analogs is the main subject of discussion in the food sector for several decades due to augmented concern related to the health impacts and sustainable development of meat

alternatives. It is, therefore, important to understand the findings of the research focused on the development, improvement, need, sustainability and functionality of meat analogs to design future strategy in this area of research. Accordingly, this paper provides a detailed discussion about the importance of developing meat analogs, health and environmental concerns of manufacturing meat products, sources of plant proteins for developing meat analogs, functionality of the ingredients used for developing these products, gastrointestinal behavior and consume attitudes related to the plant-based meat alternatives. The prime aspects to be kept under consideration for effective production of sustainable plant-based meat alternatives include requirement of suitable processing technologies, improvement in the sensory and physico-chemical functionality, safety and quality control, and exploring the gastrointestinal fate. Although the consumer acceptability of meat analogs is not widespread globally, however, a gradual improvement has been recorded recently. Therefore, future strategies should also be designed to increase the consumer acceptance of plant-based meat analogs by raising the consumer awareness and improving the quality of plant-based meat analogs to improve the functionality of these novel food products.

CRedit authorship contribution statement

Anum Ishaq: Writing – original draft, Writing – review & editing. **Shafeeqa Irfan:** Writing – original draft, Writing – review & editing. **Arooba Sameen:** Writing – original draft, Writing – review & editing. **Nauman Khalid:** Devise the idea, Edit the review and Finalize the final submission.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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