Chapter

Plant Proteins as Healthy, Sustainable and Integrative Meat Alternates

Satish Kumar, Vikas Kumar, Rakesh Sharma, Anna Aleena Paul, Priyanka Suthar and Rajni Saini

Abstract

Vegetarian protein diet based food industry have emerged as one of the fastest growing industries with largest than ever shelf space it has created in today's market. The rapid growth of plant protein industry is attributed to increased health awareness, economic and environmental sustainability issues of animal proteins and their nutritious, economical, and healthy food image among masses. Technological interventions like extrusion texturization has enabled the food engineers to create the imitation meat which approximates the esthetic attributes (texture, flavor, and appearance, binding ability, chewiness, firmness or softness) and/or chemical nature of meat. These texturized plant proteins are healthier and economical meat substitutes with sufficient opportunity to manage modify or change their functional properties in accordance to specific consumer demands.

Keywords: meat proteins, protein transition, sustainability, plant proteins, innovation, plant-based meat alternatives

1. Introduction

Meat on behalf of satisfying all the basic urges of consumer as a typical textured, juicy, flavorful, chewy food is the non-vegetarians first preference which also fulfills all the nutritional requirements of the consumers too. But the negative impression tagged with meat from very beginning always questioned its consumption may be it is related with ritualistic aspects or their significant effect on the environment due to an inefficient consumption of energy and land along with the emission of gases by meat production units [1]. The organizations and the policy makers which are involved with the sustainable production and consumption are expecting to make a shift towards more sustainable products for the consumers. However, certain products can be tagged as an alternative to the market meat which covers very less proportion of the market, called as meat replacers or meat substitutes. To overcome the dilemma of entire situation for the production of sustainable food to fulfill the needs and requirements of meat lovers the food researchers and processors formulated "meat analogue" providing higher or at least same nutritional and health benefits along with the satisfaction of meat consumption. Analogues are the structurally similar compounds slightly differs from one another on the basis of composition, in the case of meat, analogue are the products structurally similar to meat but differs

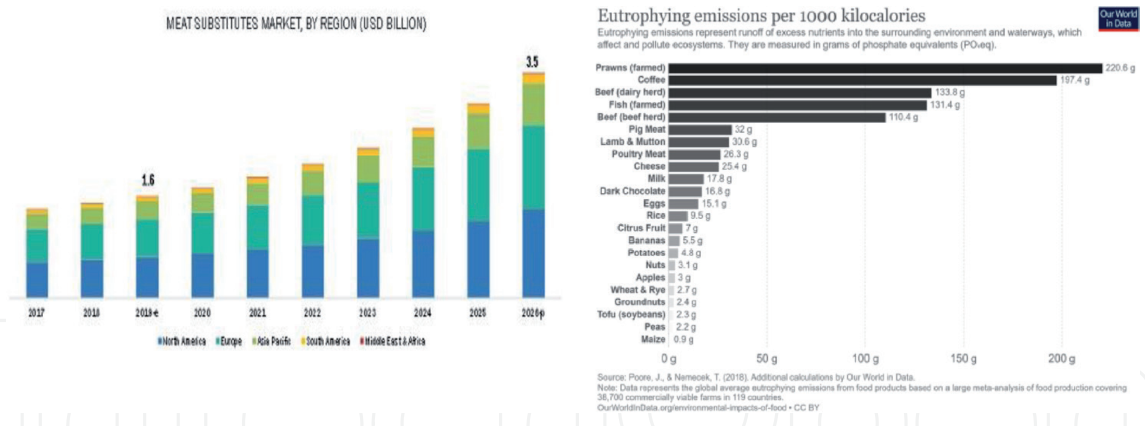

Figure 1.
Growth of meat substitutes in different continents and the eutrophying emissions of various food commodities.

in composition. They are also known as mock meat, imitation meat or faux meat approaches the qualities (mainly flavor, texture and appearance) esthetically present in meat and also fulfills the chemical characteristic of particular meat type. Such as "surimi" which is a meat-based, less expensive/healthier alternative particularly to a meat product. A general meaning of meat analogue is the food constituting non-meat ingredients sometimes without any inclusion of dairy products however, fulfilling all the nutritional and characteristic requirement of actual meat product. The meat analogue covers the maximum market which includes either vegans, vegetarians and the non-vegetarians who are in urge of reduction of meat consumption either for ethical or for health reasons and also includes people with dietary laws based on religion, such as Buddhist, Halal and Kashrut which is expected to be increasing in the upcoming years according to the data given in **Figure 1** globally the meat substitutes market was assessed to account for USD 1.6 billion in 2019 and is projected to reach USD 3.5 billion by 2026, recording a CAGR of 12.0% during the forecast period. The market is primarily driven by the increasing demand for plant-based meat products among the millennials.

The base of some vegetarian meat analogues is built on the recipes which are older than centuries viz., rice, wheat gluten, legumes, pressed tofu or tempeh with flavor addition which make them to taste like beef, chicken, ham, lamb, seafood, sausage, etc. Another meat analogue based on soybean constituting boiled soy milk layered by a thin skin is called as Yuba. Some more recent developments in soy based meat replacers is TVP (Textured Soy Proteins) derived from dry bulk commodity from soy, myco-protein-based quorn excludes vegans because it contains egg white which acts as a binder. Meat analogue functions as a meat replacer in the diet. The market does not only include vegetarian population but also include non-vegetarians who are in the urge of reducing their meat consumption either due to ethical or health perspectives. These innovations are the advantageous to the people who are concerned about the health related problems arises with the fat, cholesterol and salt overconsumption. There is also a need to develop new ways for the fulfillment of the nutritional requirements of poor at a minimum cost. Thousands of plant proteins are fortunately available in the world, explored or yet to be explored for the production of meat alternatives.

2. Driving forces for the development of meat analogue

Although the meat has a rich nutrient composition which includes essential nutrients like proteins, micronutrients such as zinc, iron and vitamin B_{12}, but taking sufficient intake of these nutrients is possible without taking meat in the diet with the consumption of variety of other foods available. In western countries, where

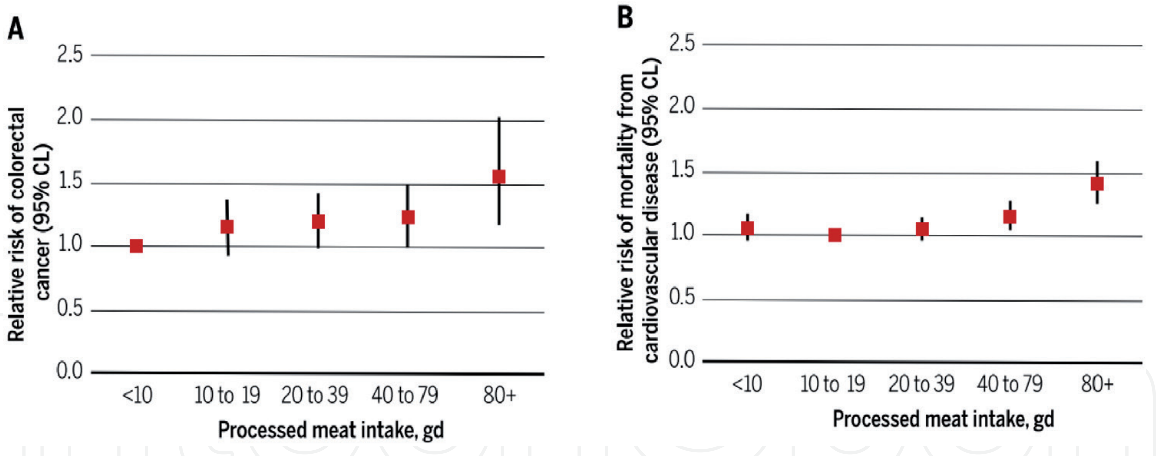

Figure 2.
Incidence of colorectal cancer (A) and development of cardiovascular diseases associated with red meat consumption.

various meta-analyses and prospective studies had undergone shows a moderately high mortality rate than usual in participants consuming more amount of processed and red meat. High intake of processed meat increases the risk of colorectal cancer is the strongest evidence of the adverse effects. The relative risk of colorectal cancer [2] and cardiovascular deaths [3] as a function of average processed meat intake shown in **Figure 2**. The International Agency for Research on Cancer (IARC) under World Health Organization categorized red meat and processed meat, due to its association with colorectal cancer probably being carcinogenic to human being [4]. According to an estimation by IARC 34,000 deaths due to cancer worldwide per year are attributable to diet high in processed meats and if the associated reports with the eating patterns of red meat were taken casual, 50,000 cancer deaths could be possible worldwide per year due to the high intake of red meat [5]. In Western Europe, the processed meat average intake is 26.4 g/day [6] will lead to an increase in the risk of colorectal cancer upto 9%. As compared to plant based foods meat produces more per unit energy emissions because there is a loss of energy at each trophic level. Within the types, the energy emission increases with poultry, mammals and ruminants production. As a reason for methane production, meat is a single most important source, which is known for its high warming potential, significantly low half-life as compared to CO_2 in the environment [7]. Grassland system careful management contributes to the storage of carbon, but it cannot be considered as a profitable effort. The use of fresh water in the agriculture field is anyhow more than any other human activity, in which the one third portions is required for the livestock, so in the areas where there is less water availability meat production is a major competitor with the other water use which also includes the maintenance of natural eco system. Production of meat is an important source of phosphorous, nitrogen and the cause of other pollutants that affects the biodiversity particularly through conversion of land to pasture and arable feed crops. **Figure 2** represents the nutrition runoff into the surrounding environment and waterways which pollute ecosystem, where meat and meat products shows maximum pollution rate.

3. Comparative overview: plat proteins vs. animal proteins

3.1 Economy and sustainability

From our ancestral period onwards, meat plays a distinct role among other food sources. Comparatively high protein content, energy that are readily available, palatability – are some major reasons for meat to rule over the diet of non-vegetarian consumers from pre-historic period. Various studies and surveys are conducted to note the increasing demand of meat in near future. It is expected that for 6 billion people around the world, in the year 2000, about 229 billion Kg of various animal products where produced; this will be doubled (approximately 465 billion Kg) by the year 2050 for 9.1 billion population [8, 9]. This hike in demand is not only attributable to the increasing population, but also to global economic developments including industrialization, urbanization, and rise in income, where another study illustrates that by the year 2030, meat consumption will be 72% more than the current statistics. Livestock farms were used previously to achieve the necessity of meat production [10, 11]. Today, limited natural resources like land and water and other ethical issues are some of the inevitable challenges for the sustainability of livestock farms.

In present scenario, about 20% electricity from total energy generated and 70% of water from total freshwater consumption is utilized for the production of food crops. Majority of thus produced crops and its wastes are used for the growth of livestocks in the form of feed and fodder, i.e., converting plant protein to animal protein for human consumption. Briefly, the reality is, when the world is facing food security and scarcity of natural resources, we are wasting majority valuable resources for the conversion plant nutrients to animal products. For instance, a study states that out of total grain and soy produced, 40% of grain and 75% of soy is used as feed. Then again, ideology of people matters a lot in order to put an end to these disputes. Studies conducted in various parts of the world give a common sketch that mostly, consumers who are health concerned is rarely diverting to the meat substitutes, and surveys shows that female population and educated people are more interested to substitutes animal protein than male population and less-educated counterparts. Factors such as awareness of the negative health impacts of meat products, comparatively lowering number of neophobic people (who shows an extreme or irrational dislike towards anything unfamiliar or new to them), transition to plant-based diet, and publics who endorse to protect and care nature are some of the reasons for the growth and sustainability of meat substitutes; indirectly through which economy and sustainable development of a nation is influenced [12].

3.2 Nutritional and functional properties

Consumption of plant and animal products always depends upon many reasons. One among them is their nutritional profile. From macro-nutrients like carbohydrates, protein and fat to micro-nutrients like vitamins and minerals varies drastically in both the sources. Due to the reason of palatability, it is commonly observed that consumption rate of animal products, including dairy and egg are always higher than the plant products. Concepts of higher biological value of egg, whey, etc. than the proteins of soy and other plant sources are also an inevitable factor for increase in the consumption of animal or meat products.

From past decades, researches have provided sufficient evidences to increasing health risksdue to meat consumption.Animal proteins are usually associated with SFAs and plant proteins with fiber and phenolic compounds. This is usually reffered as "protein package" or "whole food package". Studies conducted among no-vegetarians, pesco-vegetarians, and vegetarians show that meat consumers are observed with higher energy intake along with high quantity of SFAs, vitamin B_{12}, vitamin D, zinc, and iodine, while fiber, PUFAs, vitamin C, vitamin E are in lower concentration. Higher intake of nutrients and components like heme, cholesterol, etc. from animal sources, especially red meat, can cause to cancer, type-2 diabetes, stroke, heart disease, and metabolic syndromes. On the other hand, plant protein, regardless of the sources, are consistently related to nutrient adequacy.

Studies on substituting animal protein with plant protein are conducted widely and some of the results include: about 21% of diabetes risk was minimized on substituting processed meat, where dairy product showed no great change [13]; red meat and other high glycemic foods such as refined grains, potatoes, etc. account for high risks; purified protein (e.g. soy protein isolates) ingredients on higher consumption (~35 g/d) has a negative impact on blood lipids and blood pressure, while lower dose of soy protein (say, < 25 g/d) shows no remarkable effect [14]. Thus, both animal and plant proteins has their own influence over the consumers, where quantity and type of nutrient are important.

However, predictably, plant proteins generally show some health benefits especially against cardio-metabolic risks and other chronic diseases. This is possible by replacing many components associated with animal protein like saturated fatty acids with plant protein package. Also, inclusion of higher quantity of plant source helps in consuming fiber which has prebiotic effects, thus assisting the beneficial micro flora in gastro-intestinal tracts. Additionally, amino acids also has influence on various diseases to great extent. For example, arginine acts as a vector in vascular homeostasis. Various studies concluded that non-indispensableamino acids (arginine, glycine, cysteine andglutamine/glutamate) with potential benefits are mainly seen in plant proteins.

4. Production technology

Invasion of plant-based substitutes in our daily diet has been started decades before, whether it is milk analog or meat analog. Many of the foods we consume today consists of plant protein and had been used as a source of plant-based protein long-ago to substitute and reduce the consumption rate of meat and meat-derived products. Some of the traditional recipes include mushrooms, legumes, tempeh, wheat gluten, and pressed tofu in which slight alterations are made sometimes by adding flavors to obtain final product that can successfully imitate the meat (lamb, chicken, beef, sausage, ham, etc.) in sensory attributes. Enlightenment on the health benefits of plant-based protein over the various adverse effect of meat consumption caused a major impact on increased production rate of meat substitutes in the food market around the world. Thus many innovative foods that can replace meat in table are available easily. Generally, the production of meat substitutes in supply chain is broadly classified into four major steps: [15, 16].

i. Protein crops are identified with better quality and cultivated globally,

ii. Crops are undergone various processing methods to obtain protein ingredients such as protein isolates and concentrates,

iii. These protein ingredients are then formulated and processed into texturized intermediary products and final meat substitutes are developed,

iv. Products are distributed among consumers through retail and different food service method.

Various processing technologies are utilized for the conversion of plant-based protein into a product that can imitate meat especially in the area of texture and taste. Conceptualization of technological development in food sector is differentiated as First generation meat substitutes (mainly composed of low-moisture cooking developing intermediate products) and Second generation meat substitutes (based on high-moisture cooking extrusion, started in European markets at early period of 2000s). First generation meat substitute was found to be existing since 1990s in European markets and includes product like Textured or Texturized Vegetable Protein (TVP). The method is based on the working principle of extrusion consisting screw system within a barrel. In this method, high temperature is provided to the raw material and compressed inside the barrel, which is then conveyed through dye(s) so that an expanded final structure is obtained. This undergoes further processing to form final meat substitute. On the other hand, second generation meat substitutes were a success due to the advancement in food sectors, especially in the areas of cooking extrusion technology, various innovations in hydrocolloids and so on. These technologies opened a new insight to food market, not just because they helped in developing products that are more appealing and similar to meat, but also application of wide range of raw materials categorized as cereals, pulses, legumes, oilseeds, and aquatic plants. **Table 1** briefly explains some commonly used protein sources and its corresponding market products along with health benefits and challenges. Based on the availability, cost, convenience, nutritional profile of both raw material and final product, functional characteristics and physiological properties, numerous meat analogues are developed and are still in its experimental stages [25]. Acceptance of the final product of a meat substitute, however, has a vast influence on the ingredients used while manufacturing. **Figure 3** gives an idea on general classification on the ingredients for developing a meat analog.

Primary ingredients or protein sources	Meat substitute products	Health benefits	Challenges	References
Soy bean	Tofu, tempeh, Texturized soy protein (TSP) Schnitzel	• Reduce of chronic illnesses (CVD, immune disorders, diabetes, obesity, anti-hypersensitive andbreast and prostate cancer • Nutraceutical effects • Anti-fibrosis, anti-estrogen • Shows positive effect on thyroid and fertility	• Development of hormone-responsive tissues are reported • Beany flavor reduces the consumer acceptance	[17, 18]
Wheat	Seitan	• Helps in bowel movement • Increase gut microbiota • Decrease of serum cholesterol levels • Reduce post-prandial blood glucose level • Lowers the risk of cancer and colitis	• Gluten is a poor source of essential lysine and threonine • Many of wheat proteins are recognized as allergens and triggers of celiac disease (CD)	[19, 20]

Primary ingredients or protein sources	Meat substitute products	Health benefits	Challenges	References
Legumes (pea, lentil, lupin, chickpea)	Lupin steak and other similar products	• Gastrointestinal and gut health • Antioxidant, anti-inflammatory, anti-cancerous and cardio-protective activities • Obesity and weight management	• Presence of antinutrients and some toxic compounds like phytic acids,lectins, saponins,alkaloids, tannins and enzyme inhibitors decreases protein digestibility and bioavailability of minerals	[20]
Oilseeds	CanolaPRO™	• Reduce plasma cholesterol level by inhibiting intestinal absorption cholesterol • Decrease cell proliferation • Anti-inflammatory, antioxidative, and anticarcinogenic activities	-	[20]
Filamentous fungus *Fusarium venenatum*	Mycoproteins Quorn™ products (e.g. Meat free sausages, Meat-free chicken and Apple sausages)	• Low fat content and high protein content helps in lowering blood cholesterol level, and has glycemic properties	• Increase of uric acid level in blood leads to urolithiasis • Mycotoxins and allergies in some cases are still a challenge • Acceptance on the basis of sensory attributes like taste and color	[21, 22]
Algae *Arthrospira platensis* (spirulina)	Sausages	• Pigments like Fucoxanthin present in brown algae helps in apoptosis in human cancer cell line • anti-inflammatory, Antidiabetic, antioxidant properties • Eicosapentaenoic (EPA) &docosahexaenoic acids (DHA) are against chronic diseases	• Consumer response to green protein products due to chlorophyll • Requires complex and effective processing methods • Waste water generated may cause hazards like algae parks	[23, 24]

Table 1.
Table presenting different protein sources, corresponding meat substitutes and challenges faced for its acceptance.

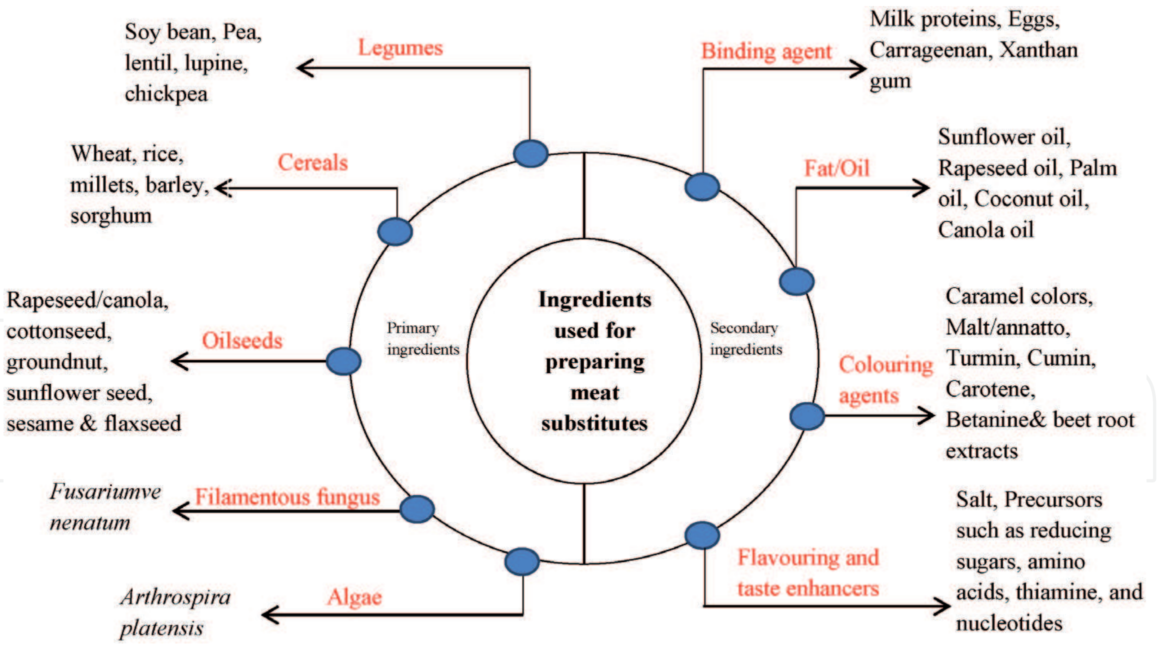

Figure 3.
Schematic representation of ingredients used in meat analog industry.

5. Common meat – substitutes available in the market

5.1 Soy-based products

Soy is considered as one of the raw material used traditionally for the preparation of various meat substitutes. From soy flour to soy protein concentrate and isolate, they have acquired quiet a significant position among the recipes. Among different soy proteins ingredients, its isolates are not only highly pure, but also has a light color with bland flavor, which makes them more approachable in product development, even though degree of purity does not play much role in the meat-analog applications. Properties like water-holding capacity, gelling property, fat-absorbing capacity, emulsifying capacity and other functional properties of soy ingredients makes them more reliable in this industry and are available in numerous forms like toasted flour, full-fat, de-fatted (about 50% of protein content and are produced from grinding defatted soy flakes), etc. By fractionating defatted soy flakes, soy protein concentrates (protein content is found to be 70%) and isolates (protein content is 90%) are obtained through aqueous alcohol extraction and alkaline extraction followed by precipitation in acidic pH, respectively [26].

5.2 Tofu

Soybean derived tofu or soy curd, being an excellent source of protein and minerals like iron and calcium, is the mostly utilized meat substitute world-wide and are available in block form. Production of tofu is said to be simple, clean, convenient and controllable process. Nutritional profile of tofu includes 8% of total proteins, 2% of carbohydrates and lipids about 4 to 5% on fresh weight basis. Absence of cholesterol, low energy value, high amount of vitamins and minerals and presence of dietary fibers (about 1%) are some of the relevant factors for the high demand of soy curd. Traditionally, tofu is prepared by protein coagulation of hot soy milk with the assistance of a coagulant: salt-induced($CaSO_4$, $CaCl_2$or $MgCl_2$) or acid-induced

(Glucono Delta-Lactone (GDL) – also known as gluconolactone) to obtain a gel-like product. Some studies says that coagulant used for the production of tofu plays a vital role in determining its quality; yet controversies are still existing.

The complex process starts from soaking and grinding of soybeans followed by filtering to extract the milk. Then it undergoes methods like boiling, coagulation, breaking of curd, pressing and reforming the gel. However, packed or filled tofu involves addition of coagulant to the cooled soy milk and is then heated and coagulated in a suitable package, without undergoing any further processing methods. Some studies says that, presence of isoflavones, oligosaccharides, trypsin inhibitors in soymilk cause allergic reactions, off-flavor and flatulence, respectively in some people. As a result, soy protein isolate (SPI) has gained a considerable demand as raw material for packed tofu, as it has better health benefits to consumers. Apart from coagulant used, some other influential factors in tofu-making are the processing conditions, concentration of coagulant used, and two major storage proteins: glycinin (11S) and β-conglycinin (7S) [27].

5.3 Tempeh

Tempeh, a traditional soy fermented product, is considered as the food that can provide the most health benefits among other soy products and higher consumption percentage in many places. Countries like Indonesia (70% of households), Australia, China, Japan, and Taiwan and also in some places of Europe, America and Africa are reported to have higher consumption of tempeh. Presence of high quantity of crude protein and essential amino acids, fatty acids, carbohydrates, folic acid (416.4 μg/100 mg), isoflavones, vitamin B_{12} (3.9 μg/100 g), and tocopherols (α-, β-, γ- and δ-), along with reduction of antinutritional factors such asphytates, saponins, trypsin inhibitors, hemagglutinins, and flatus factors, with increased facilitating, detoxification properties, bioavailability of minerals and many otherhealth benefits, tempeh is considered as a better choice for healthy diet [28]. Presence of umami taste (a basic taste that can be detected by human receptors) is also said to be a factor for its increased demand.

Tempeh, in general, is a collective name for combination of cooked and fermented raw material (cereals, beans or a byproduct of food processing) with any suitable culture ofmycelium of a living molds, yeasts, lactic acid bacteria (LAB) or various gram-negative bacteria. Even though *Rhizopus oligosporus*is the dominant microorganism used for the production of tempeh, molds like *R. oryzae* and *Mucorspp* are also used for enhancing flavor, texture and nutritional characteristics.

Production of tempeh involves acidification of soybeans using lactic acid or acetic acid to inhibit the growth of undesired microorganisms by lowering the pH (5 or below), followed by boiling and cooling (30–38°C). Inoculation at 25–30°C process is carried out and a compact, creamy, white, fresh tempeh cake will be resulted after 1–2 days. Due to the production of different proteases secreted by *R. microsporus var. oligosporus*such as 'aspartic acid protease' or known as 'acidic protease' and another endopeptidase called 'serine protease' helps to break complex soy proteins at aspartic acid residues (when at 3–4 pH) and at small/side chains like glycine and alanine residue (at neutral to alkaline pH, i.e. 7–11), respectively. However, for commercial purpose, mixed cultures are also used for better and quality yield [29]. In addition, fermentation process not only helps to improvise the nutritional and sensory profile of the final product, but also imparts health benefits including enhanced antioxidant property, and plays a role in fighting dementia, cardiovascular diseases andcancer (especially, colorectal cancer and hormone-depended cancers like breast cancer and prostate cancer) [28].

5.4 Textured soy protein(TSP)/soy meat

In food industries, inorder to reduce the rate of saturated fat and cholesterol consumption, vegetable proteins (VP) are incorporated into food, increasing the protein and essential amino acid content. Sources of all vegetable proteins like legumes (protein content is maximum, varying from 25 to 50%), nuts and soy are used for the production of Textured Vegetable Protein (TVP), which is found in fibrous, insoluble and porous form and considered as an excellent meat analog or meat substitute. TVP is otherwise known as textured soy protein (TSP) or soy meat, where proteins present in defatted soy flour is concentrated, isolated and extruded based on 'extrusion cooking', providing better taste and chewy-texture when compared to meat or seafood.Soy meat has about 50% protein content, which is found to be decreasing on rehydration.This method was primarily adopted by many Asian countries. TVP was commercially developed in America and by late 1960s, it was successfully welcomed by European markets.

Soy meat is presently considered as an economic option for replacing meat (e.g. meatballs) by both vegetarians and health conscious consumers. TSP is used as a meat extender in many products, thus replacing 30% of meat without affecting the sensory characteristics. For instance, in some parts of the world, quantity of beef in samosa stuffing has been replaced around 50% with granules from defatted soy flour with no major observable difference in sensory attributes. Production of TVP starts from washing of the selected soybeans and is soaked at 30°C for 3 hrs in order to remove antinutritional factors by softening the husk. Soaked beans are then washed till the husks are completely removed and are dried at 70°C for 5 to 8 hrs. TSP is developed through 'hot extrusion' where different dyes are used for producing high protein nuggets, chunks, etc. Today, soy meat is expanding worldwide rapidly, especially in the developed countries. Since soy meat is considered as "poor man's food" by many, it is a reliable source of protein for under-developed countries and low-income people [30].

5.5 Cereal-based products

Cereals comprise of nine species (wheat, rice, corn, barley, sorghum, millet, oat, rye, and triticale) under the family *Gramineae.* In meat analog industry, cereals are mainly used for extending the meat products. For example, 3–9% of quail meat roll is extended with corn flour resulting better emulsifying stability with yield. Similarly, chicken patties are also substituted with 10% barley flour, 5% sorghum and 5% pressed rice, which has no significant change in its sensory profile. Studies with rice and barnyard millet includes the decreased level of cholesterol and increased magnesium content among the consumers and use of these cereals has also not shown any negative impression on color, except barnyard millet slightly affect the flavor of developed meat substitute. Today industries are substituting cereals 9% or less, which is more convenient. Thus, cereals play a vital role as an important ingredient, particularly as a meat extender. In some cases, combination of cereals gives better yield and quality to the product, without adversely affecting its sensory. On the other hand, presence of gluten in cereals like wheat, oats, barley and rye arise question among the consumers due to its potential of allergy [31].

5.6 Seitan

Wheat protein has been playing a vital role in human diet from thousands of years ago. Traditionally, they were used widely in the regions of Japan, Korea, China and Russia, to replace meat products. Wheat being a common cereal used in majority of countries, it is possible to rely on wheat-based meat substitutes, especially in regional level. "Wheat meat" or "wheat gluten" is developed from the component called gluten (wheat proteins -gliadin and glutenin), which is easily extracted through a simple procedure of rinsing with water in order to remove starch and bran. As a result, a chewy mass is obtained, which can be further processed with additives and cooking methods to attain wheat-based meat substitute. This simple, conventional and economic method is utilized by many food industries that deal with vegetarian burgers, sausages, minced meat, nuggets and schnitzel. Mostly, rinsed out starch is utilized as byproduct for other purposes, thus reducing the food waste. When gluten is flavored by simmering in a broth of soy sauce, garlic and ginger to obtain seitan, which has small quantity of sodium. Gluten has the capacity to form a thin film on elongation resulting a natural stringy fibrous proteinaceous structure seitan, which helps it to imitate the texture and consistency remarkably. Seitan is not just an alternative for the non-vegetarian diet, but also it is an ideal choice for people who are reluctant to consume soy products. Even though many nations like Western countries started to use seitan as a part of their food, some Arab countries are not in favorable due to its unpleasant flavor, failing the sensory attributes [32].

5.7 Mycoproteins

Apart from plant-based proteins, scientists diverted their experiments towards single-cell proteins (SCP) for the development of meat substitutes. SCP can be described as protein derived from pure or mixed cultures of microbes such as bacteria, yeast, fungi or microalgae. Most common source of single-cell protein is the filamentous fungi, which produce mycoproteins when grown under specific controlled environments inside a bioreactor. Processes like forming, steaming and subsequently texturizing are done to achieve finished products. Sometimes, for obtaining desired products, binding agents like egg albumin and flavoring agents are also used accordingly. Mycoprotein is also known as 'fungal protein' and described as "Generally Recognized as Safe". Commercially, *Fusarium (F.) venenatum* is widely used in food industries for the production of mycoproteins and industrially known as Quorn™.

Studies on fungal mycelium for substituting meat were started during 1960s by Rank Hovis McDougall (RHM), a British company. After fruitfully completing the development of product and its toxicity testing, first product was launched in the year 1985 with the approval from British Ministry of Agriculture. Thereafter, many researches are revolving around mycoproteins. For instance, a study on the biological value of mycoproteins was found similar to the milk proteins and toxicology study says that these fungi derived proteins have no harmful effects on human beings and animals [33]. Additionally, it provides some health benefits to the consumers suffering from various chronic diseases. Presence of protein (45%), carbohydrates (10%), fat (13%), fibers (25%) on dry basis along with various vitamins and minerals helps them to replace several meat products in our food basket. Also, studies have proved that intake of mycoproteins have a positive effect on lowering total and LDL (low-density lipoprotein) cholesterol and increasing HDL (high-density lipoprotein) cholesterol level in blood; appetite regulation as short-chain fatty acids (SCFAs) produced as a result of gut digestion of mycoprotein fibers send satiation signals from colon to brain; presence of soluble fiber not only helps to slow down the diffusion of glucose molecules through the intestinal walls, but also decreases the rate of absorption of glucose, thereby having a positive outcome on glycemic condition; and they can fight against food-borne pathogens which are commonly seen in numerous meat products [21].

Marlow Foods, developer of Quorn, develops mycoprotein through continuous fermentation of desired culture accompanied by glucose and other required nutrients and oxygenated water. In US market, about fifteen Quorn products are sold, among which products: Meat-free sausages, Meat-free chicken and Apple sausages are refrigerated items [23]. Production method of mycoprotein is said to have a favorable influence on environmental issues also. That is, they can reduce the amount of greenhouse gas emission from agriculture and food allied sectors, use of innumerous fertilizers, antibiotics and pesticides can be decreased to a large extend, wastage of land and water and nutrient-cycle recycling, especially reactive nitrogen species (RNS) - NH_4^+, NO_3^- and NO_2^- by fixing nitrogen gas. However, many regulations are adopted by many nations due to some negative impressions of mycoproteins. Firstly, presence of higher concentration of nucleic acid (NA) in mycoproteins can cause urolithiasis due to increase of uric acid level in blood. Secondly, Use of microalgae and yeast can affect sensory attributes like flavor and color of final product. Thirdly, verifications regarding mycotoxins are inevitable as a part of safety precautions. Lastly, reports on gastrointestinal tract (GIT) reactions, sometimes leads to life-threatening allergies (anaphylaxis and urticarial) [21].

6. Miscellaneous ingredients and their role

6.1 Binding agents

To develop a product that can mimic another is always a challenge. Especially to satisfy its sensory appeal. Additives that are capable of binding water and fat are important while developing a plant-based meat substitute. These ingredients can either be the plant source itself such as isolate or concentrate of soy protein and wheat gluten or some external components like egg, xanthan gum, milk protein or carrageenan. These ingredients, which are having high protein content, majorly functions as water binder and forms protein networks. On the other hand, some ingredients with no protein content (e.g. starch, soy flour, etc.) are used as fillers taking into consideration of their binding properties by entrapping water and fat physically.

However, to attain a quality product, concentration of binding agent used at the time of manufacturing of meat analog is vital. Industries make use of gluten due to its cohesive nature, leavening ability, dough forming ability and visco-elastic nature. In addition to these properties, gluten is profitable while processing because of the reduced cooking loss and can improve slicing attributes [34, 35]. In some case, polysaccharides (e.g. guar gum, pectin, cellulose, carrageenan, etc.) are used as binders. The gelling and thickening properties helps polysaccharides to improve the rheological properties of developed product. Hydrocolloids such as xanthan gum, starches, pectin and locust bean gum are found to have a positive effect on sausages and other similar products which are low in fat content [36]. Other than the application of egg albumin, soy ingredients (flour, concentrates and isolates) are also extensively used. However, beany taste of soy products makes the industries to limit their application to isolates [26].

6.2 Fat/oil

One of the prime objectives of developing meat analog is to reduce the cholesterol level to minimum. As a result, today, meat-substitute products available in the market contain comparatively low fat. Another concern is that, quantity of fat can affect fiber structure and its formation during processing undesirably. Studies reported similar cases, for instance, during extrusion process, dough that have oil content more than 15% was failure as the lubrication of the material affected the alignment of its macromolecules; and also the slippery texture negatively affect the shear force exerted at the time of extrusion [37, 38]. Although raw materials are fatted before using them for manufacturing meat substitutes, plant sources like soy, oilseeds, etc. has natural oil present in them. This quantity is more or less sufficient in many cases. However, for increasing the sensory attributes, industries use some additives such as soy oil, sunflower oil, rapeseed oil, canola oil, palm oil, corn oil, coconut oil, etc. These added oils give the final plant-based meat analogues a juicy and tender texture, along with release of flavor and retaining the volatile components [26].

6.3 Flavoring agents and taste enhancers

Flavor is one of the most important sensory attribute for food as it gives a satiety before even consumption. And in the case of meat products, flavors arising from the product itself make consumers to stick around the non-vegetarian foods. Therefore, while preparing a meat analog, ensuring the customers with similar flavor in essential. In many products, fat/oil is added extra, which can also serve as a precursor for flavoring by entrapping the organoleptic volatile components. Addition of flavoring agents is hence common in meat analog industry. These agents improve the acceptability of product not only by mimicking the flavors of meat products, but also by lowering the beany flavors generated (e.g. beany flavor developed for soy-based meat substitutes). A study conducted on mushroom concentrates for replacing monosodium glutamate or hydrolysed vegetable protein was effective when formulated at less than 1%. Also, simple sugars and sulfur-containing amino acids have strong impact on developing flavor, while other compounds like glycoprotein, monosodium glutamate, etc. helps to mask pungent sulphury taste or improves the meaty flavor [10, 11].

However, during extrusion process (or while undergoing treatments at high temperature and pressure), it undergoes various physiochemical changes, making the whole process a complex. This results in loss of natural and added flavors like spices and other agents. Conversely, application of high temperature give rise to reactions like Maillard reaction, where amino acid and sugars present in the matter creates a distinctive flavor. This improves sensory characteristics – flavor and taste, even though it is risky if not optimized properly by reason of the generation of off-flavor. Among numerous aromas available in meat products, roast and smoked aromas are desired by many consumers. Additionally, several studies shown that furans and thiophenes containing sulfur or sulfur-containing heterocyclic compounds imparts strong meaty strong meaty-roasty-flavor to finished products [26].

6.4 Coloring agents

In meat products, degree of color change and color characteristic are very important, hence it is essential to maintain same color attributes in meat analogues. This can be achieved by incorporating edible additives which imparts desired color in final product. For example, protein from soy and wheat (gluten) possess yellow-brow color and is much brighter than original cooked meat and very different from raw meat color. Edible food color are used such caramel, annatto, carotene, turmin and cumin as they are heat stable. Other colors may be used in meat analogue are beet root and betanine extracts and reducing sugars for browning characteristics in Millard reaction in presence of protein. Such reducing sugars are mannose, lactose, xylose, arabinose, dextrose and maltose. In general, coloring solutions are added with proteins before extrusion. But still majorly meat analogues have poor color quality due to improper balance of pH between meat alternative products and color solution. This issue can be resolve by addition of appropriate acids alone or in combination like acetic acid, citric acid and lactic acids. Also, color retention agents like hydrated alginate or maltodextrin can be added to control the color migration from meat alternative products [26].

6.5 Advantages and disadvantages

The expanding world's population facing the problem due to lack of food led to hunger issues along with preserving ecosystem. In 20th century, the drastic nutritional transition was developed and presents livestock as a major available protein source globally. However, consumption of animal products also reported with various serious food safety issues.

Meat and poultry products are popular and consumed all over the world but many ethical, traditional and environment issues forced the production of meat analogues. Plant based many products were designed recently to substitute the conventional meat products. The basic criteria of protein for the meat analogue are essential such as oil and water holding capacity. During the slaughtering of animals, food animals were reared which is a conversion of vegetable protein to meat protein. This process required many resources like water, fodder, feed and so on. Also, crop production required low inputs comparatively. Thus, production of meat reported with inefficient compare to plant based meat analogue. As per Pimentel and Pimentel [39], meat production required more water in comparison with plant crops (approximately 100 times more). The requirement of water in human diets, for meat diets per person in a year was the double than the vegan diets per person in a year. Meat products greatly depend on meat analogue which further vary with consumer acceptability, cost and legal criteria of the country. Along with this, the current living style associated with health diseases encourages the production of plant based meat analogue. Broadly the major concerns with meat products are classified as environmental issues, animal welfare issues and public issues. The expenditure of huge amount of natural resources and emission of greenhouse gases by the meat productions enhances the ecological burden which led to deforestation also. Animal welfare issues involve the unethically torturing and slaughtering process. This sometimes led to forceful and cruel transportation of animals. Public health issues comes in picture when the over consumption of meat reported resulting in development of ischemic heart disease and cause of 1.8 million deaths every year [40]. Larsson and Wolk [41] reported the colorectal cancer risk associated with consumption of red meat (120 g red meat per day or 30 g processed meat/day). *Salmonella, Campylobacter* and *E.coli* pathogen outbreaks reported several times all over the world which is also found in meat products. These major issues raise the food safety and public health concerns and studies. Meat analogue have advantage of producing various shape and size like sheets, cubes, disk etc. with desired color.

In terms of nutrition and sensory, still no plant based meat product was successfully developed which can completely replace the meat products. The plant based meat substitute lacks with the similar mouthfeel due to the hardy and rubbery texture of meat analogues. The other drawback reported for the plant based meat analogue is off-flavors. Aforementioned, sensory attributes of plant based meat analogue still lacking leading to the undesirable flavors in the end product. Due some allergic reactions, not all plants can be used for production of meat analogues which is an important health concern. Major disadvantage for the meat analogue is related to its production process which involves 4 steps starting from cultivation of plant crop followed by protein concentration. Further, formulation and texturizing of meat substitute and marketing add up the various unit operations. In comparison with animal meat, few unit operations are required.

6.6 Laws and regulations

The health importance in protein in diet is scientifically documented and in 1999, FDA (Food and Drug Administration, U.S.A.) also recognized soy protein as blood cholesterol ingredient when consume 25 g every day when included in regular diet. This statement led to utilization of plant proteins in different health and function food products [42]. In 2016, GPA (Green Protein Alliance: multi-stakeholder platform with other partners) in Netherlands, focused on protein consumption balance to 50:50 from 63:37(animal:plant sources) by 2025 [15, 16]. In a study, an American consume 112 g protein (77 g protein from animal source and 35 g plant source) in daily diet and RDA for protein in adult is 56 g from both type of diet (vegan and non-vegan). These data conclude that consumption of protein is twice than RDA [39]. Incorporation of plant based protein in meat analogue related products will be helpful in balancing the proteins by following standardizing the nutrient allowance.

6.7 Future prospectus and challenges

The real challenge for the production of meat analogue is to attain same sensory attributes like taste, texture, smell, aroma, etc.). Different food researcher and scientist introduced various techniques such as extrusion and shearing were used to produce desired texture. Another challenge was discussed is in terms of nutritional profile and only 30% protein was reported in meat alternatives. However, Protein Digestibility Corrected Amino Acid Score (PDCAAS) should be focused further for complete replacement of meat analogues with meat products. Not only plant, but other source of meat analogue like microbes (bacteria, yeast, algae) and mushrooms can be used which may fit with the human diet and consumer taste. But maximum attention can be devoted to improve mouth-feel properties meat substitutes which is low in commercially available meat analogue. The large scale production of meat analogue still a challenge until the low sensory attributes of meat substitutes solved and thereby, it will take time to shift the purchasing habits of consumers. In market terms, future of meat analogues is safe and demand is increasing by passing year. For now, meat substitute are viable only for to those vegan diet depended consumers. Presently, the willingness of consuming meat analogue is between low to moderate and availability of low varieties of meat alternates influence its market success, although demand is growing every year. For all protein producers to have better connectivity to consumers there will need to be better investment in systems for efficiency and quality, overlaid with welfare, environmental and health standards.

7. Conclusion

There is a high demand of meat analogue based on plant protein which is growing every year. The development of meat alternative is useful not only to vegan consuming diet but also focus in the environment sustainability and consumer health safety prospective with higher or at least similar benefits as animal meat products. But still the availability and variety of plant based meat analogue is very limited in global market which may be due to several issues. One of the major concerns is how to get the exact texture and flavor in the meat substitutes, and this has resulted in development of the Umami taste. Furthermore, issues related with production of meat analogues like mouth-feel is biggest barrier in the acceptance and replacement of real meat products from the market. Another point is that cultured meat products are not promoted in markets till date due to its complex production procedures and unreported health concerns and their safety issues. The production of meat analogue using plant protein can be considered as a safer and sustainable way to provide the health conscious people an alternate to balance the recommended dietary allowance specially for the proteins without the concerns of the saturate fat intakes. Further, the market trends suggests that there is a huge potential of such innovative food materials in the market which is further expected to grow in the near future as some of the big players of the food processing sector are investing heavily in this sector for the potential improvement in the existing products or the development of the new innovative products for all.

Acknowledgements

The authors are thankful to Lovely Professional University Phagwara, Punjab Agricultural University Ludhiana and Dr. YS. Parmar University of Horticulture and Forestry Solan for providing infrastructure and financial support for the study.

Conflict of interest

The authors declare that they have no conflict of interest.

Author details

Satish Kumar[1]*, Vikas Kumar[2], Rakesh Sharma[3], Anna Aleena Paul[1], Priyanka Suthar[1] and Rajni Saini[1]

1 Food Technology and Nutrition, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India

2 Department of Food Science and Technology, Punjab Agricultural University, Ludhiana, Punjab, India

3 Department of Food Science and Technology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, HP, India

*Address all correspondence to: satishsharma1666@gmail.com

References

[1] McMichael AJ, Powles JW, Butler CD, and Uauy R. (2007). Food, livestock production, energy, climate change, and health. Lancet, 370, 1253-1263.

[2] Norat, T., Bingham, S., Ferrari, P., Slimani, N., Jenab, M., Mazuir, M., Overvad, K., Olsen, A., Tjønneland, A., Clavel, F. and Boutron-Ruault, M.C., 2005. Meat, fish, and colorectal cancer risk: The European prospective investigation into cancer and nutrition. Journal of the National Cancer Institute, *97*(12), pp.906-916.

[3] Rohrmann, S., Overvad, K., Bueno-de-Mesquita, H.B., Jakobsen, M.U., Egeberg, R., Tjønneland, A., Nailler, L., Boutron-Ruault, M.C., Clavel-Chapelon, F., Krogh, V. and Palli, D., 2013. Meat consumption and mortality-results from the European prospective investigation into cancer and nutrition. BMC Medicine, *11*(1), p.63.

[4] Bouvard, V., Loomis, D., Guyton, K.Z., Grosse, Y., Ghissassi, F.E., Benbrahim-Tallaa, L., Guha, N., Mattock, H., Straif, K. and Corpet, D., 2015. Carcinogenicity of consumption of red and processed meat. The Lancet Oncology, 16(16), pp.1599-1160

[5] IARC (The International Agency for Research on Cancer). 2015. World Health Organization, Q&A on the carcinogenicity of the consumption of red meat and processed meat. *2015-10-29)[2015-12-02]. http://www. cancer. ie/content/qa-carcinogenicityconsumption-red-meat-and-processed-meat# sthash. iz4J5AxV. dpbs*.

[6] Micha, R., Khatibzadeh, S., Shi, P., Andrews, K.G., Engell, R.E. and Mozaffarian, D., Chronic Diseases Expert, G.(2015). Global, regional and national consumption of major food groups in 1990 and 2010: A systematic analysis including 266 country-specific nutrition surveys worldwide. *British Medical Journal*, *5*(9), p.e008705.

[7] Ismail, I., Hwang, Y.H. and Joo, S.T., 2020. Meat analog as future food: A review. Journal of Animal Science and Technology, *62*(2), p.111.

[8] Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M. and De Haan, C. (2006). In: Livestock's long shadow: Environmental issues and options. Rome, Italy: FAO. FAO 978-92-5-195571-7

[9] Bruinsma, J. (2009). The resource outlook to 2050: By how much do land, water and crop yields need to increase by 2050? Rome, Italy: FAO. In: Expert Meeting on How to feed the World in 2050. http://www.fao.org/wsfs/forum2050.

[10] Kumar, P., Chatli, M. K., Mehta, N., Singh, P., Malav, O. P., &Verma, A. K. (2017a). Meat analogues: Health promising sustainable meat substitutes. Critical Reviews in Food Science and Nutrition, *57*(5), 923-932.

[11] Kumar, P., Chatli, M. K., Mehta, N., Singh, P., Malav, O. P., &Verma, A. K. (2017b). Meat analogues: Health promising sustainable meat substitutes. Critical Reviews in Food Science and Nutrition, *57*(5), 923-932.

[12] Siegrist, M., & Hartmann, C. (2019). Impact of sustainability perception on consumption of organic meat and meat substitutes. Appetite, *132*, 196-202.

[13] Malik, V. S., Li, Y., Tobias, D. K., Pan, A., & Hu, F. B. (2016). Dietary protein intake and risk of type 2 diabetes in US men and women. American Journal of Epidemiology, *183*(8), 715-728.

[14] Mariotti, F. (2019). Animal and plant protein sources and cardiometabolic health. Advances in Nutrition, *10*(Supplement_4), S351-S366.

[15] Tziva, M., Negro, S. O., Kalfagianni, A., &Hekkert, M. P. (2020a). Understanding the protein transition: The rise of plant-based meat substitutes. Environmental Innovation and Societal Transitions, *35*, 217-231.

[16] Tziva, M., Negro, S. O., Kalfagianni, A., &Hekkert, M. P. (2020b). Understanding the protein transition: The rise of plant-based meat substitutes. Environmental Innovation and Societal Transitions, *35*, 217-231.

[17] Zaheer, K., &Humayoun Akhtar, M. (2017). An updated review of dietary isoflavones: Nutrition, processing, bioavailability and impacts on human health. Critical Reviews in Food Science and Nutrition, *57*(6), 1280-1293.

[18] Dukariya, G., Shah, S., Singh, G., & Kumar, A. (2020). Soybean and its products: Nutritional and health benefits. J Nut Sci Heal Diet, *1*(2), 22-29.

[19] Scherf, K. A. (2019). Immunoreactive cereal proteins in wheat allergy, non-celiac gluten/wheat sensitivity (NCGS) and celiac disease. Current Opinion in Food Science, *25*, 35-41.

[20] Albuquerque, T. G., Nunes, M. A., Bessada, S. M., Costa, H. S., & Oliveira, M. B. P. (2020). Biologically active and health promoting food components of nuts, oilseeds, fruits, vegetables, cereals, and legumes. In *Chemical Analysis of Food* (pp. 609-656). Academic Press.

[21] Hashempour-Baltork, F., Khosravi-Darani, K., Hosseini, H., Farshi, P., &Reihani, S. F. S. (2020). Mycoproteins as safe meat substitutes. Journal of Cleaner Production, *253*, 119958.

[22] Souza Filho, P. F., Andersson, D., Ferreira, J. A., &Taherzadeh, M. J. (2019). Mycoprotein: Environmental impact and health aspects. World Journal of Microbiology and Biotechnology, *35*(10), 147.

[23] Ajwalia, R. (2020). Meat alternative gaining importance over traditional meat products: A review. Food and Agriculture Spectrum Journal, *1*(2).

[24] Ibañez, E., Herrero, M., Mendiola, J. A., & Castro-Puyana, M. (2012). Extraction and characterization of bioactive compounds with health benefits from marine resources: macro and micro algae, cyanobacteria, and invertebrates. In *Marine bioactive compounds* (pp. 55-98). Springer, Boston, MA.

[25] Smetana, S., Mathys, A., Knoch, A., & Heinz, V. (2015). Meat alternatives: Life cycle assessment of most known meat substitutes. The International Journal of Life Cycle Assessment, *20*(9), 1254-1267.

[26] Kyriakopoulou, K., Dekkers, B., & van der Goot, A. J. (2019). Plant-based meat analogues. In *Sustainable meat production and processing* (pp. 103-126). Academic Press.

[27] Wang, X., Luo, K., Liu, S., Zeng, M., Adhikari, B., He, Z., & Chen, J. (2018). Textural and rheological properties of soy protein isolate tofu-type emulsion gels: Influence of soybean variety and coagulant type. Food Biophysics, *13*(3), 324-332.

[28] Mani, V., & Ming, L. C. (2017). Tempeh and other fermented soybean products rich in isoflavones. In *Fermented foods in health and disease prevention* (pp. 453-474). Academic Press.

[29] Amin, M. N. G., Kusnadi, J., Hsu, J. L., Doerksen, R. J., & Huang, T. C. (2020). Identification of a novel umami peptide in tempeh (Indonesian fermented soybean) and its binding mechanism to the umami receptor T1R. *Food Chemistry*, 127411.

[30] Alamu, E. O., &Busie, M. D. (2019). Effect of textured soy protein (TSP) inclusion on the sensory characteristics and acceptability of local dishes in Nigeria. Cogent Food & Agriculture, *5*(1), 1671749.

[31] Pintado, T., & Delgado-Pando, G. (2020). Towards more sustainable meat products: Extenders as a way of reducing meat content. Food, *9*(8), 1044.

[32] Anwar, D., &Ghadir, E. C. (2019). Nutritional quality, amino acid profiles, protein digestibility corrected amino acid scores and antioxidant properties of fried tofu and seitan. Food and Environment Safety Journal, *18*(3).

[33] Finnigan, T., Needham, L., & Abbott, C. (2017). Mycoprotein: a healthy new protein with a low environmental impact. In *Sustainable protein sources* (pp. 305-325). Academic Press.

[34] Malav, O. P., Talukder, S., Gokulakrishnan, P., & Chand, S. (2015). Meat analog: A review. Critical Reviews in Food Science and Nutrition, *55*(9), 1241-1245.

[35] Asgar, M. A., Fazilah, A., Huda, N., Bhat, R., & Karim, A. A. (2010). Nonmeat protein alternatives as meat extenders and meat analogs. Comprehensive Reviews in Food Science and Food Safety, *9*(5), 513-529.

[36] Arora, B., Kamal, S., & Sharma, V. P. (2017). Effect of binding agents on quality characteristics of mushroom based sausage analogue. Journal of Food Processing and Preservation, *41*(5), e13134.

[37] Gwiazda, S., Noguchi, A., &Saio, K. (1987). Microstructural studies of texturized vegetable protein products: Effects of oil addition and transformation of raw materials in various sections of a twin screw extruder. Food Structure, *6*(1), 8.

[38] Cheftel, J. C., Kitagawa, M., &Queguiner, C. (1992). New protein texturization processes by extrusion cooking at high moisture levels. Food Reviews International, *8*(2), 235-275.

[39] Pimentel, D., & Pimentel, M. (2003). Sustainability of meat-based and plant-based diets and the environment. The American Journal of Clinical Nutrition, *78*(3), 660S–663S.

[40] Key, T. J., Davey, G. K., & Appleby, P. N. (1999). Health benefits of a vegetarian diet. Proceedings of the Nutrition Society, *58*(2), 271-275.

[41] Larsson, S. C., &Wolk, A. (2006). Meat consumption and risk of colorectal cancer: A meta-analysis of prospective studies. International Journal of Cancer, *119*(11), 2657-2664.

[42] Cho, M. J. (2010). Soy protein functionality and food bar texture. In *Chemistry, texture, and flavor of soy* (pp. 293-319). American Chemical Society.