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Plant-based (Vegan) Meat

Pankti Patel and Sury Pratap Singh

Abstract
Search for meat substitute is currently trending. Animal -based foods are being replaced by plant-based foods in people diet. Plant-based sources are inexpensive, have a high nutritious content, environment friendly and have a low danger of spreading any zoonotic diseases. Soy bean has been used as the foundational ingredients for vegan meat preparation. Lentils, mushroom extract, texturized vegetable protein (TVP) are added as auxiliary ingredients to provide nutritional value and increase sensory characteristics. Structuring technologies used includes top-down strategy and bottom-up strategy. Future-generation youths are more likely to favor environmentally beneficial technologies. This paper summaries all the aspects of preparation and analysis of vegan meat.

Keywords: Plant-based meat analogue, plant protein and vegan meat etc.

Introduction

Vegetarian individuals are people who avoid eating any meat, poultry or fish. They are further sub-divided as lacto-ovo vegetarians and vegans. Lacto-ovo vegetarians consume dairy products and/or eggs while vegan strictly eat plant-based diet. Egg-vegetarian are those who eat eggs and pescatarians are those who eat fish in their diet [89]. Flexitarian are semi-vegetarians who eat meat occasionally. A well-planned diet of vegetarians and non-vegetarians were compared and studied which showed distinct changes in their nutrient uptake. Vegetarian diet is rich in carbohydrates, n-6 fatty acids, dietary fiber, carotenoids, folic acid, vitamin C, vitamin E and Mg but low in protein, saturated fat, long-chain n-3 fatty acids, retinol, vitamin B12 and Zn. Vegans faced low intakes of vitamin B12 and calcium [20]. Hence people move towards non -vegetarian source. Meat refers to muscle tissue of slaughter animal (edible parts) removed from carcass [43]. Meat is popular for its unique chewy texture, juicy and its rich meaty flavour along with all nutrients [81]. The meaty taste and odor are to some extent due to formation of lactic acid (breakdown of glycogen) and organic compounds like amino acids, dipeptides and tripe tides broken from meat protein. Sometimes to enhance meat flavour MSG (monosodium glutamate) about 0.05-0.1% is added [43]. Meat substitutes can be defined as meat analogues which mimic textural and sensory characteristics like original meat. It is also known as mock meat or faux meat [52, 60]. Soy and gluten are the two widely used protein as meat substitutes. Amino acids content and characteristics of protein makes soy a decent substitute for meat [118]. Soy is related to genetically modified (GM) crops which makes it unacceptable among many consumers. According to research, approximately 60% of people don't believe GM scientists and 55% of consumers oppose eating GM foods [25]. Wheat protein gluten guarantees good network in the product, but some individuals may avoid using due to its allergy. Meat alternatives may include protein isolates, concentrates and powder extracted or derived from plant. Focusing on its nutritional properties, any food product which exceeds 30% protein content with low fat is considered good meat alternative [65]. Sources must be added for supplementation of iron and vit B12 which is actually present in meat.

Traditionally tofu, tempeh, seitan etc. were used as alternative to substitute with real meat. The recent development focuses on development of meat analogue not only adjusting the nutritional requirements but also focuses on physical and sensory characteristics. Meat products available are strips, chunks, patties and burger, chicken-like blocks, ground beef-like products, nuggets, steaks, sausages etc. [65]. Extrusion, shearing, spinning, and freeze alignment are popular techniques currently used to texturize vegetable proteins from oilseeds, pulses and grains generating a variety of forms, while fermentation is traditionally used for the development of mycoprotein. Extrusion and mixing are most frequently used while some techniques are still under developmental stage.

Structuring technologies includes top-down approaches in which dough is formed into structured product and bottom-up approaches in which individual fibers are prepared and organized to resemble meat [23, 66, 120]. Plant based meat is less popular as customer find them as highly processed food.

Protein in human diet

It is important to realize that our body must digest the protein in order to provide with proper nutrition. For dietary protein to be utilized by the body, it must first be digested in the gastrointestinal system and get absorbed through the intestine. Proteases enzyme hydrolyze food protein in the stomach and small intestine to produce big peptides and finally to short peptides and unbound amino acids. The components of protein digestion in the small intestinal lumen include approximately 20% of free amino acids, 80% of dipeptides, and additional tripe tides. The small intestine's lumen the dietary amino acids are catabolized by luminal bacteria and some are oxidized by enterocytes. 60% of free amino acids are transported by Na+ dependent system and 40% is transported by Na+ independent amino acid system respectively to sustain growth, development, reproduction, lactation and health, humans need dietary proteins which are the main source of these nitrogenous nutrients. However, producing significant quantities of these crystalline protein is quite expensive. Hence protein has to be supplemented through plant and animal- based food products [141].

Nutritional Composition of Protein Source

Meat: Meat is rich source of protein and micronutrients like iron, selenium, zinc and vitamin B12 [9]. Recommended Dietary Allowance (RDA) of red meat is about 25% consisting of riboflavin (vit B2), niacin (vit B3), pyridoxine (vit B6), and pantothenic acid (vit B5) per 100 g [140]. Protein digestibility score can be estimated by Protein Digestibility-Corrected Amino Acid Scores (PDCAAS). Protein quality or PDCAAS method is a measure of protein ability to provide enough level of essential amino acids for human need. Score is calculated using an amino acids profile and true digestibility of a food protein [39]. Score 1.00 is for egg white and casein proteins. Score for meat is 0.92, while pinto beans, lentils, pea and chickpea score between 0.57 to 0.71. Wheat gluten scores 0.25 [3].

Soybean: Soybean (*Glycine max*) also called soja bean or soya bean belongs to *Fabaceae* family. Soy bean is high quality protein with PDCAAS score 1.0 [39]. Soy protein is most preferred substitute due to its high nutritional value, extending properties and binding characteristics. Soybeans are good source of fibers and nutrients too. Both consumption of dry beans and soybeans have shown protective and therapeutic effects. Genistein and daidzein are two is flavones found in soy. It also offers healthy promoting effects include lowering cholesterol, preserving bone density, improving vascular health and easing menopause symptoms. Soybeans are low in carbohydrates (35%). The principal soluble carbohydrates of raw soybeans are the sucrose, raff nose and stachyose. Soybean contains roughly ~19% oil, of which the triglycerides are the major component. Soy oil is characterized by relatively large amounts of the polyunsaturated fatty acids (PUFA) i.e., ~51% linoleic acid, ~8% α-linolenic acid, stearic acid ~4, palmitic acid ~10, oleic acid ~23 of total fatty acids [2].

Quinoa: Quinoa (*Chenopodium quinoa*) is pseudo-cereal belonging to *Chenopodiaceous* family and genus *Chenopodium*. Pseudo-cereals are cereals high in protein, starchy seeds and free from gluten. All 9 essential amino acids and 8% of protein are present in quinoa. The addition of quinoa as meat substitute increases its ability to retain water, reduces its toughness and enhances its sensory characteristics [34, 93]. In quinoa, albumin and globulin makes up 44%-77% of total protein, which is more than prolamin 0.5-7.0%. Due to its negligible or total absent of prolamin makes quinoa gluten-free cereal. Quinoa is a tasty, nutritious and easy to prepare food good for individuals with gluten intolerance, such as those who have celiac disease. Fat percentage in quinoa ranges between 2% to 10%. Quinoa and soya oils have similar fatty acid compositions. Quinoa is rich in essential fatty acids such as linoleic acid (18:2 n-6:52%) and linolenic acid (18:3 n-6:40%). Compared to other cereals, it includes more calcium, magnesium, iron and zinc. Quinoa seeds lose 27% of their copper and 3% of magnesium content when they are washed and polished, which results in 12-15% reduction in concentration of iron, zinc and potassium. Compared to rice, barley and wheat, it contains higher riboflavin and α-tocopherol. It contains good amount of vitamin E. The drawback of using quinoa is presence of saponins and phytic acid. Trypsin inhibitor and tannins are present in less amount. Trypsin inhibitor are inactivated by heat treatment as they are thermo labile compounds. Polyphenols (tannins) are present in trace amounts (0.53 g/100 g in the whole quinoa seeds) which are further decreased during scrubbing and washing with water (0.23 g/100 g). It can be served as a hot breakfast cereal or popped like popcorn or sprouted to use as salad topping. Quinoa flour can be mixed with maize or wheat flour and used as substitution in bread (10–13% quinoa flour), noodles and pasta (30–40% quinoa flour) and sweet biscuits (60% quinoa flour) [131].

Wheat: Wheat belongs to genus *Triticum*. Wheat contributes 20% of the food's calories and approximately 55% of the carbohydrate. It has significant content of vitamins and minerals, including thiamine and vitamin B, as well as carbohydrates 78.10%, protein 14.70%, fat 2.10%, and minerals like zinc and iron up to 2.10%. Additionally, wheat is a significant source of trace elements like selenium and magnesium nutrients crucial for overall health [1, 36, 121, 130].

Oats: Oats (*Avena sativa L.*) is gaining much attention due to its high nutritional value and health benefits. Oats is typically processed as whole grains either directly rolled as flakes or grounded into flour. Oats are good substitute for gluten free products for celiac patients. Carbohydrates are the main constituents of oats (58.7 g/ 100 g) mainly contains starch, few sugars and oligosaccharides. It contains relatively high level of dietary fiber (9 g/ 100g) and protein (14g / 100g). Oats have relatively high lipid content linolenic acid (2.4g) and α- linolenic acid (0.11g). Oats protein has distinct amino acid content like globulin (Avenal in), albumin, prolamin and glut Elin. These are further categorized on basis of their solubility stated by Osborne fractionation [55, 142]. Oats consists of saline-soluble globulins (50–80%) of total oat proteins whereas the alcohol-soluble prolamin (4–15%) of the total protein [27, 122]. Albumin and glut Elin fractions have high level of tryptophan. Oats serves as good source of vit-E [135].

Legumes: Legumes belongs to *Fabaceae* family [105]. The fruit or its pods are edible when they attain maturity. Legumes are highly nutritious and many antioxidants are also present. Depending on its variety and environmental conditions their protein content range from 20% to 35%. They are rich source of dietary fiber, vitamins, and minerals like magnesium, iron, zinc, potassium and phosphorus [26]. They are low in saturated fat and free from cholesterol [61, 75]. Legumes have high proportion of lysine and threonine but lacks methionine, cystine and tryptophan as compared to cereals. Albumin and globulin are two portions of the protein found in legume seeds. Albumins are water soluble; comprises 10–25% of the total protein, and are present in the germinal portion. The nutritional value of a seed increases with albumin content. Globulins are soluble in diluted neutral salt solutions [69].

Oil Seeds: Oil seeds are good source of protein and contain sulphur containing amino acids. They have antioxidant, antihypertensive and neuroprotective properties. The oil plants used as source of protein are inter alia, soybean, chia seeds, evening primrose, flaxseed (brown), hemp seeds, milk thistle, nigella seeds, pumpkin seeds, rapeseed, sesame, safflower, glandless cottonseed, and sunflower seeds [57].

Table 1: Merits and Demerits of Vegetarian diet

Merits of Vegetarian Diet	Demerits of Vegetarian Diet
Higher amount of dietary fiber rich in antioxidants water content higher less saturated fat	Deficient of zinc, vitamin B12, iron less amount of EPA + DHA source biological value of protein less

Table 2: Merits and Demerits of Meat

Merits of Meat in Diet	Demerits of Meat in Diet
Most effective source of iron and zinc. Optimum Source of Vit-B complex, mainly B12 Biological value of protein higher	Higher fat content in some portion of meat Higher sodium content in processed meat

Source: Summing up about meat and vegetarian diet [97]

Composition of Plant-Based Meat

The biggest problem is to replicate the unique flavors and textures of real meat in vegan meat. A meat analogue contains water (50%-80%), textured vegetable proteins (10%-25%), non-textured proteins (4%-20%), flavorings (3%-10%), fat (0%-15%), binging agents (1%-5%) and coloring agents (0%-0.5%) [29]. The high water content in the product lowers its costs and give product the desired juiciness acting as a plasticizer during processing, and assisting in emulsification. The ideal texture, mouth feel, and appearance is enhanced by using textured proteins and thus increasing its nutritional purpose. Texturized proteins can be used in place of meat to replace the two ways: by blending the texturized proteins with meat (i.e., meat extension) or complete replacement of meat by texturized proteins to form fully vegan or vegetarian products [108]. The water-holding capacity, texture and emulsification qualities of the product are adjusted together with the processing parameters by adding soy protein isolates and concentrates, wheat gluten, egg white and other binding agents such hydrocolloids and starches. Consumer acceptance plays major role. However, the amount of fat added to the product can also have an impact on the final food product's aroma, colour and texture. The major role of meat replacer is to give flavor and mouth feel without any meat component [47, 108, 124].

Shifting Towards Novel Protein Sources

One of the most crucial elements to make meat analogues is protein. Proteins play crucial roles in the hydration, solubility, emulsification, foaming, flavour binding, viscosity, gelation, texturization and dough formation processes [114].

Soy Protein: Soybean consists of mixture of water soluble and insoluble proteins. Soybean on acidification at *p*H 4.5-4.8, proteins get separated into storage globulin and whey fractions. According to sedimentation coefficient, globular proteins are classified into four protein categories 2S, 7S, 11S, and 15S. 80% of protein comprises of 7S (β-conglycinin) and 11S (glycinin) [90]. Soy protein isolates and concentrates are used in making sausage, burger and meat muscles like meat alternative. Soy derived products like soy milk and soy flour are used for making protein rich ingredients. Tofu is made by coagulation of soymilk and is rich source of protein, calcium and iron. It makes gel like structure which takes up all flavor during cooking. Tempeh is another soy derived product where cooked soybean and grains like rice and millet are fermented using *Rhizopus oligoporus* as culture organism. Both tempeh and tofu are prepared from soya bean hence gives meatier taste [123]. Yuba, a soy-based alternative meat replacement, is prepared by layering the thin skin that forms on top of cooked soy milk [46, 47].

Wheat Protein: Wheat Gluten the byproduct of wheat starch plays important role as meat alternative. It functions as binding agent, dough formation and provide viscosity. Gluten is extracted by washing of soluble components with water and remaining is insoluble protein [8, 22]. During extrusion or shear cell process, gluten present in dough changes into fibrous structuring giving it meat like texture and used in making whole-cut and minced type alternative [59, 99]. Fibrous structure formation during high- extrusion process [13, 100, 133] leads to formation 3D cross link of disulphide protein [92].

Yeast Protein: Impossible Foods (Founder: Patrick O. Brown) one of its products is plant-based burger (PBB), which is a replacement created to mimic the taste and preparation of ground beef. Texturized wheat protein (wheat TVP), coconut oil and potato protein are the main components of PBB. The company created a modified yeast culture to produce "HEME" (soy leg hemoglobin), a naturally occurring protein in the root nodules of leguminous plants that serves as an analogue for the myoglobin that gives beef its distinctive flavour and cooking characteristics. This protein gives beef its characteristic flavour and cooking properties. Comparing to the resources used by Animal Farming and real meat burger, Impossible burger utilizes 75% less water, 87% less emission of greenhouse gas and 95 %less land than conventional ground beef [33, 37, 125].

Fungus Protein: In 1985, UK launched a novel plant-based meat product “Quorn meat”. The interesting part is they used soil fungus *Fusarium* to obtain its mycoprotein by fermentation [137]. The mycoprotein obtained has less fat content with more protein and fiber content. This happened because fungus was rich in amino acids [4]. The fungus was cultured under controlled condition in bioreactor and then further processed for forming, steaming and texturizing [137].

Legume Protein: Legume protein is obtained from pea, lupin, lentil, faba bean, mung bean and chick pea etc. Pea protein can be considered as versatile legume protein due to its ability to bind water and fat to get firm texture withstanding thermal processing [88]. Functionality and usefulness of peas depends on cultivation, extraction process and composition (legumin/vicilin ratio) [18]. Desired functionality of legume protein can be achieved by applying post processing thermal treatment, [10, 96] using mixture of ingredients [68] or using less refined protein rich ingredients [73, 85, 144].

Seed Protein: Seed isolates and concentrates protein are obtained as byproduct of oil industry. There use as protein source could be great utilization process but presence of antinutritional factors like polyphenols limits its use [35]. Salgado, *et al.* (2012) noted that sunflower protein concentrates possess moderate water holding capacity and can be used as thickening agent [115]. Sunflower concentrates can also act as gelling agent. Ultra sonication of sunflower isolates at 95 °C result in formation of stronger gel which was further increased by n lowering temperature till 25 °C [74]. Rapeseed consists of crucifer in (11S globulin) and napin (1.7-2S albumin) protein present can gel under high temperature and pressure which gives meat like texture [44, 50]. It can be used in making sausage type meat alternative. For chia seeds, less refined fraction has highest emulsifying stability while protein enrich fraction has greater protein solubility, water absorption and lesser gelling capacity [15].

Algal Protein: Microalgae can create up to 70% of the proteins in cells, compared to 30-40% for soybeans, depending on the strain and culture circumstances. *Spirulina (Arthrospira)* and *Chlorella* are the most popular microalgae species with 102.6 and 107.5 essential amino acid index which is higher than average index 100 [54].

Insect Protein: Abundant protein is present in insects with average 40% protein content and ranging between 20%-70% depending on species used. *Tenebrio molitor* with 52.35% protein, *Gryllodes sigillatus* with 70% protein and *Schistocerca gregaria* with 76% protein are the most widely used insect species in Europe [147]. Within these same species, amount of protein and its quality differs depending on its diet, metamorphic stage and kind of habitat [49]. Protein digestibility can be described as: Animal Protein (95% egg protein, 98% beef protein) > Insect Protein (76%-98%) > Plant Protein (lentils 52%) [58]. Insect proteins are rich threonine and lysine content but poor source of methionine or tryptophan. Insect-derived proteins have a low level of solubility that ranges from 3% to 45% which may be increased by enzymatic hydrolysis [103].

Other Protein Sources: Other protein rich source include peanut, potato protein, hemp and corn zein fraction

Other Essential Ingredients

Binding and Texturizing Agents: Binders are substances added to bind water and fat and help TVP particle to stick together. Arora *et al.* (2017) noted that use f carrageenan and xanthan gum improved textural properties of mushroom-based sausage analogue prepared with 5% saturated fat [7]. Starches and flours used to improve product consistency and texture can be classified as a type of carbohydrate ingredient. Binding agents and gums, such as methylcellulose, acacia gum, xanthan gum, carrageenan, and many others are also defined as a type of carbohydrate ingredient [60, 65, 104].

Lipid Source: Conventional meat analogue products have low levels of lipids than new generation meat analogue. Different lipid ingredients like fats and oils are employed in the creation of meat analogues similar to protein ingredients. Canola oil, rapeseed oil, coconut oil, sunflower oil, corn oil, sesame oil, cocoa butter and many more types of vegetable and plant oils are used. Review by Kyriakopoulou, *et al.* (2019) stated that fats and oils in meat analogue formulations play a part in the product's juiciness, tenderness, mouthfeel, and flavour release. However, careful attention should be paid to how the fats and oils are impacted during processing and preparation to avoid excessive lubrication and stickiness [65].

Flavoring Agents: The rich flavor of cooked meat is due to extreme complex decomposition, oxidation, and reduction reaction. Reactions included are Millard Reaction between amino acids and reducing sugars, oxidation of fatty acid and thermal degradation of thiamine [110]. Soy protein isolate imparts off-flavor such as beany, grassy, chalky, bitter and astringent taste [146]. It is due to presence of is flavones and saponins [62]. Flavor of meat substitute is divided into distinct phase. In initial phase, on meat protein is enzymatically hydrolyzed to create taste of meat. In second phase, is to vegetable oil is oxidized for getting lipid flavor, finally natural spices reduce off-flavor and odor to encourage flavor [143]. Yeast extract is used as flavor enhancer as it contains precursors like reducing sugar, amino acids, peptides, nucleotides, lipids and thiamine which contribute meaty flavor when heated [5]. Alliin a percussor to flavor compound that give garlic its distinctive flavor was extracted in 1948 by Stoll and Seebeck using ethanol as solvent. Alliin can degrade into alkyl disulphide such as dially trisulfide, diallyl sulfide and dially disulfide giving meat like flavor [67, 143]. Salt is common flavor enhancer and increases the shelf life of product. The addition of salt to protein base meat analogue cause protein stabilization and unfolding properties. Protein isolates a contain some amount of salt during fractional process [95]. High level of salt intake may lead to ill health [56].

Coloring Agents: Coloring agents are pigments either chemically synthesized or naturally extracted or as dry powder. To simulate the red colour of beef, coloring additives such annatto extracts (E 160b), lycopene, beet juice extract or leg hemoglobin are used. Other components are added to ensure the heat stability of these pigments such as ascorbic acid or juices high in polyphenols. They act as antibacterial and preservative substances and improves quality stability and shelf-life extension [42, 118].

Fortifying ingredients: Tocopherol, zincglyconate, thiamine hydrochloride, iron, zinc, sodium ascorbate, niacin, pyridoxine hydrochloride, riboflavin and cobalamin are naturally found in red meat. Plant-based meat is fortified with minerals, vitamins and amino acids [19].

Water: Water serves several purposes in meat analogue. During processing it serves as hydration medium for various dried ingredients as well as plasticizer and reaction agent. Water content determines viscosity of melt, takes parts in chemical reaction and acts as medium for energy transfer in extrusion processing [145].

Structuring Process: Bottom-up strategy

Cultured Meat: Myoblast cells are harvested from skeletal muscles of animals and are allowed to multiply in serum supplemented medium rich in nutrients like amino acids, lipids, vitamins and salts. Muscle fibers are ready to harvest after three weeks approximately. These fibers are less than 1 mm thick and 2-3 cm long. As a proof of concept, these muscle fibers have been employed to create one hamburger [101].

Mycoprotein: Quorn meat substitutes, uses mycoprotein using the filamentous fungus *Fusarium venenatum* as a base. For fermentation process, bioreactor conditions are adjusted which is ideal for the growth of the fungus like temperature, pH which must be closely monitored and regulated. After fermentation, the RNA must undergo a heat treatment to break it down into monomers and centrifugation to create a paste-like product with 20% solids from the leftover biomass. After centrifugation further processing includes forming, steaming, chilling, and texturizing required to obtain fibrous products. Commercially mycoproteins are used for minced-type products like chunks, sausages, and burgers [137, 138].

Spinning Process: Based on biopolymer solution alignment, spinning process can be divided into two types as wet spinning and electro spinning. In 1954, Boyer received a patent for wet spinning of proteins for the use of meat analogue [112]. Through the use of a spinneret, a protein-containing solution is extruded and then submerged in a non-protein-solvent bath. Exchange between solvent and no solvent causes the extruded protein phase to precipitate and solidify, resulting in stretched filaments with a thickness of about 20 m [106, 112. 128]. In electro spinning, biopolymer solution is forced through a hollow needle or spinneret, which has an electric potential in relation to a ground electrode. Surface instabilities create development of very thin fibers (less than 100 nm), which are drawn to the ground electrode [117]. These fibers are used as meat analogue. Electro spinning of proteins is used for whey, collagen, egg and gelatin but rarely for plant proteins [38, 48]. Proteins are hard to electro spin, hence mixed with spinnable polymers like cellulose and malt dextrin to increase efficiency of this technique [63].

Top-up strategy

Extrusion: Extrusion is most used techniques for production of TVPs. It is relatively cheap, energy efficient method with high output. TVPs are prepared with the addition of water, salts, binders, lipids, flavors and stabilizers. The raw ingredients are allowed to pass through extruder screw where under the influence of high temperature and pressure the products get its desired structure and shape. Extrusion structuring is of two types: low-moisture extrusion and high-moisture extrusion. Additionally, extrusion inactivates hydrolytic enzymes including lipoxygenases, peroxidases, and lip oxidases, denatures heat-labile antinutritional factors like trypsin inhibitors and hem agglutinins, and improves protein digestibility [53, 79]. Low-moisture extrusion processes flour or concentrates into TVP, which are dry, slightly expanded product with spongy texture are obtained, then moisturized. Fibrous meat like products with 50% wt. or more are produced by high-moisture extrusion. Inside the barrel, the protein is plasticized through combination of heating, hydration and mechanical deformation. Extrusion is operated on high temperatures (140-180 ℃) which ensures protein melting and polymerization. However, it also causes changes such as color due to Maillard reaction, caramelization, hydrolysis and degradation of pigments are downfall of this process. [11, 51, 84].

Mixing of protein and hydrocolloids: Proteins can be combined with hydrocolloids that precipitates with multivalent cation to produce fibrous products. After mixing, the fibrous products are washed and the excess water is pressed out, and dry matter obtained ranges between 40-60% wt. [64]. Stable emulsion of colloidal solution from a mixture of water, a vegetable fat or oil with a protein like lupine protein, pea protein, potato protein or rape protein and hydrocolloids. Casein can coagulate with cations so when added to the emulsion cations to start the fiber formation process, resulting the entrapment of the anisotropic structures. By controlling the concentration of the hydrocolloids, fibrous structure formation can be controlled [32].

Freeze Structuring: Freeze structuring involves freezing of protein slurry. Heat is removed from well mixed slurry which results in isotropic structure but if slurry is heated without mixing, anisotropic structure is formed. Ice-crystal needles are formed which depends rate and freezing temperature [16, 71, 83]. High-temperature drying helps to fix the protein's fibrous texture while preventing the melting of the ice crystals. Protein textural characteristics can be adjusted by varying the freezing conditions like freezing rate, *p*H, material's solids content, surface effects, and heat exchange effects, degree of condensation and pressure effects [111].

Shear Cell Technology: The process is done either in a shear cell which is a conical device based on a cone plate remoter, or a Couette cell which present a cylindrical shape. Fibrous structure is obtained by combination of simple shear and heat [59]. The heating temperature is most important factor (140 °C) which results in a solid anisotropic food texture, whereas low temperature results in layered structure [40].

Formulation

Emulsion type Products: Products that are similar to emulsions include meat-based foods like sausages, frankfurters, bologna, Moraxella, etc. A significant portion of water, proteins, lipids, carbohydrates like gums, fibers and starch etc., salt and spices are present in plant-based products like present in animal-based meat. Many plant-based emulsion-type products were developed as a result of applications involving meat and meat extenders, in which high protein non-meat substances replace meat to some extent [23].

Burgers, Patties and Nuggets: Attempts are made to replicate the characteristic bite, chewiness and firmness of ground animal-based meat products using plant-based ingredients. The main components of meat-based burgers, patties, and nuggets are proteins and lipids, with seasoning, salt and binders such as wheat crumb, starches and fibers. Salt alters the structure of proteins and toughens products though added in less proportion [109]. Binders help the product retain water and fat thus enhancing its texture and appearance. The majority of the protein components are first converted into textured vegetable protein, fiber structure that resembles ground meat and then combined with the remaining ingredients to create the final formulation [98].

Chicken-Like and Steak-Like Products: The imitation of whole-cut meats such as chicken, pork and beef steak are distinguished by the presence of long fibers or layered structure is major goal. The method of producing plant-based goods that replicate this layered or fibrous structure is extrusion. To obtain the desired final structure, colour, softness, aroma, and flavour alteration the products are further treated by freezing, curing, marinating and cooking. Shear cell technology has the potential to produce big chunks of fibrous plant-based goods. Structuring stage is crucial to the development of both a fibrous and a juicy product [17].

Technique for Evaluating Quality Parameters of Plant-based Meat

The crucial techniques and steps that meat analogue developer would follow in standardizing their methodical approaches could be in sequence like: raw material → process → product → consumer

Table 1: Proximate composition and protein characterization

Methods Employed and Principle	Practical Perspective	References
Total Protein Content **Dumas Method:** The food sample is burned at 900–1300 °C in an O_2 rich environment, and the combusted gas is passed over a gas–water membrane to separate the non–nitrogen gas. Nitrogen-containing gas is fed via a copper tube for reduction. A thermal-conductivity detector produces an electrical signal which measures the nitrogen content from residual gas.	Calibration: standard EDTA and THAM Determined nitrogen content: 0.003mg-50 mg	[31]
Kjeldahl Method: Firstly, digestion step uses boiling sulfuric acid and a catalyst, converting the nitrogen in the samples into ammonium ions. Secondly, in distillation step the liquid is neutralized with NaOH. The ammonia is recovered by condensation and reconverted to non-volatile ammonium by using boric acid. Lastly, the amount of recovered ammonium in the borate is determined by acid titration.	Calibration: pH calibration/indictor. Determined nitrogen content: 0.02 mg	[76]
Amino Acid Content Analysis **Acid hydrolysis:** Acid hydrolysis is done where amino acids are separated in an ion-exchange HPLC system and detected with a ninhydrin post-column derivation.	For estimation of cystine and methionine these are oxidized before hydrolysis. Deamination of asparagine and glutamine is done during hydrolysis.	[72, 107, 113, 119]
Protein Profile (type and amount of different protein present) **SDS-PAGE:** In this method, denatured protein can be seen by electrophoresis. For electrophoresis, A concentration 8%-12% acrylamide gel is used for protein separation. Lastly, gel is stained with Coomassie brilliant blue to make the proteins visible.	For stabilizing charges of protein and in order to increase its solubility Laemmli buffer is used. Silver staining could be used as alternative staining method	[6, 12]
Total Fat Content **Soxhlet Extraction Method:** Samples are placed in an organic thimble and soaked in a nonpolar solvent with low boiling point (hexane or petroleum ether) which solubilizes fat. The fat is released into the solvent container and solvent is recovered through distillation. The extracted fat can be known gravimetrically.	Key step is moisture removal from analogue prior extraction. This can de done by using oven-drying.	[134]
Total Mineral Content Ash is produced after samples are heated at high temperature and burned to eliminate organic compounds.	Complete removal of organic compound is vital for precise determination. For particular mineral detection, methods used are atomic emission spectroscopy or ion chromatography	[45]
Total Fiber Content Similar to human digestion, enzymes like amylases, proteases, amyl glucosidases acts on sample further lined by precipitation and filtration which yields the high molecular weight dietary fiber succeeding protein and ash determination. The low molecular weight soluble dietary fiber left as permeate can be determined by HPLC.	-	[80]
Fast Proximate Composition Analysis Infrared Spectroscopy is rapid analysis technique used to test constitutes like water, fats and protein present in meat analogue.	-	[102, 128]
Differential Scanning Calorimetry (DSC): DSC detects phase transitions during protein denaturation or fat crystal melting. Protein denatures at specific temperature which is help to build fibrous structure. Additionally, fats and oils undergo enzymatic modifications.	Proteins are stabilized by counterbalancing covalent and non-covalent interaction which favors native conformation and denaturation.	[14, 21, 28, 132]

Table 2: Texture and Sensory Properties

Methods Employed and Principle	Practical Perspective	References
Texture profile analysis and Rheometer Double compression test: Meat analogue sample is placed between parallel plates to compress and relax to mimic a twofold chewing stroke. **Rational Rheometer:** In controlled strain Rheometer, shear to sample is employed by specified strain or rotational speed on lower plate and torque on upper plate to maintain its geometry. In controlled stress Rheometer, shear stress is generated on upper geometry while motor produce torque which is transformed into stress. Closed-cavity Rheometer use oscillatory measurements to study protein dispersion and its deformation.	Meat analogue samples should be prepared in precise to make sure equal stress is applied. Analogues should not be compressed before performing rheology. This could result in changes in protein or fat structures. Moisture loss and water holding capacity should be maintained. Sample should be covered with silicon oil before testing.	[24, 30, 77, 82, 91]
Sensory Evaluation **Affective tests:** Non-trained person or consumer's rate food according to their liking and satisfaction. 9-point hedonic scale ranging from "dislike extremely" to "like extremely" is mostly employed. **Descriptive tests:** Food description is already pre-decided and trained panel judge it.	Food products is presented to panelist in random sample order of uniform size, at specified temperature, blinded with a three-digit blinding code.	[13, 41, 70, 94, 116, 126, 127, 136]
Appearance **Color L*a*b*:** Color is measured with colorimeter and uses CIELAB color space with D65 as a standard illuminant. Colorimeters consist of pulsed xenon arc lamp which illuminates the sample and light reflected which is collected using photocells and thus calculate the coordinates in the color space. CIELAB color space, L* describes the lightness of the sample black=0 and white= 100, whereas a* ranges from green (−) to red (+), and b* ranges from blue (−) to yellow (+).	L*a*b* results should be interpreted with digital photographs of a sample to get a real data of its overall appearance making it easy for reader to analyze it.	[78, 87]
Microstructure **Scanning Electron Microscope (SEM):** SEM consists of an electron source that produces a beam of primary electrons. The electron beam focuses and demagnify	Before microscopy sample is pretreated by fixation, drying and coated to enhance its conductivity. These treatments may change	[28, 86, 118]

with help of electromagnetic lenses, and then coils are used to direct the beam across the specimen surface. Vacuum pumps generate a vacuum to prevent entrapping of air and detect it. SEM is mostly used to observe the fibrous microstructure of meat analogue. **Confocal laser Scanning Microscopy (CLSM):** The CLSM consists of laser which produces a point illumination with a pinhole and the light emitted passes through a second pinhole of focal plane and laser detects it. Fluorescence dyes Nile red and Rhoda mine B can be used to visualize protein and fats in meat analogue.	microstructure of the sample and lead to faulty result.	

Consumer acceptance towards plant-based meat

Consumer's acceptance towards plant-based meat varied among themselves looking on their age, living standards and awareness. The most attributes chargeable for its acceptance is sensory characteristics. Even they are displeased by its texture and flavour. Extruded soy protein or legume protein may impart beany flavour which is not accepted by many people. Another attribute is cost. High income countries consumed meat on regular basis on their convenience. Plant based meat is highly processed food which makes it expensive. Some non-vegetarian due to ill health associated to obesity or cardiac disease may shift toward plant-based meat. Environmental concern and animal welfare are recently trending among youths. Vegetarians and vegan avoid eating meat due to their ethical values and motivate individuals to shift towards vegan or vegetarian diet. Researchers are attempting to substitute meat with other protein sources to help people enjoy food with hurting ethical concerns [32].

Conclusion

Since people are becoming aware of negative effects of eating meat as it affects both their health and environment, the demand for plant-based meat is rising significantly. Food producers have several opportunities to convince customers who want to put limit on meat consumption. Researchers have reported that customers found plant-based meat less appealing to its bald flavour. But nowadays new techniques are used for its formation. Their main goal is to get meat like flavors and textures. This paper discusses various structuring techniques and formulation and different ingredients used to replicate meat flavor and texture to ensure customer satisfaction. The two main components utilized as meat substitute are typically soy protein and wheat protein. There is a need to identify new alternatives because soy is linked to GMO and celiac illness in people who consume gluten. Many techniques and tests as discussed above are carried out to ensure safety and evaluating nutritional value of meat analogue. Exploring novel protein alternative and deciding security and chemical safety of plant-based alternative is point of research for future finding and safety.

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