Technological Review of High-Moisture Extrusion for Creating Whole-Cut Plant-Based Meat

An overview of the state of high-moisture extrusion technology and the corresponding state of ingredient development, technical and sensory analysis, and infrastructure in India

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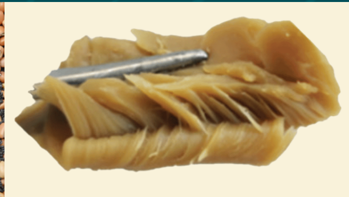


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Introduction and scope

Plant-based meat products are structured plant-derived foods designed to replace animal-based meat as stand-alone products or within recipes. Plant-based meat products closely resemble animal meat counterparts in terms of organoleptic properties (color, taste, smell, and texture), using one or a combination of alternative protein ingredients. The success of plant-based meat products relies on creating end products that match the entire sensory experience of eating traditional meat products. While various meat analogs are now available globally, many of these products are addressing the need for alternatives to minced meat like burger patties and meat-derived products like sausages and frankfurters. The most technically challenging products for current plant-based meat technologies are the whole-muscle meat analogs, which can replace chicken breasts, steaks, and salmon filets. The spatial arrangement of proteins is integral to the unique texture of animal-derived whole-muscle meat products, which impose challenges in bio-mimicking them with plant ingredients. Particularly, plant proteins have entirely different profiles than animal proteins. These differences necessitate significant technological interventions to optimize raw materials and ingredients as well as decipher and leverage structuring mechanisms that can transform plant proteins into whole-muscle meat analogs with the right texture, taste, appearance, and organoleptic properties. Owing to these complexities, whole-muscle analog products are limited and relatively less mature in the market.

Currently, several technologies are under investigation to produce plant-based whole meat analogs, which include extrusion using twin-screw extruders with cooling dies (Chiang et al., 2019), electrospinning (Nieuwland et al., 2014), and shear cell technology (Grabowska et al., 2014). Nevertheless, among these technologies, extrusion is the most widely used structuring technology to create texturized plant protein products, owing to the ease of scalability in a short time. The applications of other technologies such as shear cell, 3D printing, electrospinning, wet spinning, freeze structuring, and mechanical elongation have been limited to academic research or pilot-scale operations. Extrusion is defined as **a system of pushing mixed ingredients out through a small opening, called a die, to form and shape the materials** (Launay and Lisch 1983). The resultant formed products are termed extrudates (Berk 2009).

Over the last several decades, extruders that are conventionally used for producing texturized vegetable protein have been retrofitted to create plant-based meat analogs and animal meat extenders. Despite their versatility to handle a wide range of capacities and commercial viability, extruders require heavy capital investment, which can present barriers to entry for early-stage entrepreneurs. In India, there is a dearth of not only manufacturing-scale extruders but also pilot-scale extruders, creating a scale-up barrier for entrepreneurs who want to expand their production of plant-based meat beyond the proof-of-concept stage. Additionally, the process of creating meat analogs is not a definitive science. Researchers have actively explored new ingredient profiles and blends, analyzing the correlation between the process, system, and product parameters of extrusion. Although it is evident that the operating parameters influence the product properties by affecting the extrusion system parameters, limitations with in-line detection have rendered the extrusion process as a black box. Hence, research in this area has been evolving with an enhanced focus on process and product parameters (Thiébaud et al., 1996; Fang et al., 2014; Maung et al., 2021; Pietsch et al., 2019).

This paper is the first in a series of analyses of various plant protein structuring technologies and is focused on identifying the current state of understanding of the role of ingredients, process parameters, and equipment design in producing whole-cut meat analogs using high-moisture extrusion (HME) technology. The objective is to provide a complete overview of the state of technological advancement, the cost analysis framework for setting up an HME facility, the high-impact research problems and knowledge gaps that prevail currently, and the white space opportunities to the entrepreneurs, suppliers, and researchers who are exploring extrusion. To get an overview of the market and regulatory developments in the global plant-based meat industry, readers can refer to GFI's latest 'State of the Industry Report: Plant-Based Meat, Seafood, Eggs, and Dairy'.

Classification of meat analogs

Meat analogs can be divided into three categories (Fig. 1) based on the extent of processing and degree of deviation from the animal-derived meat. The first-generation or emulsion-type products have a unique texture and sensory profile as a result of water-holding and fat-binding capacities similar to those demonstrated by the finely chopped animal muscle proteins – myosin and actin, when solubilized in a 2-3% salt solution. Other than protein, emulsion-type products contain water, fats, gums, fibers, starch, and salt that impart structure along with spices that provide flavor. Second-generation or minced-type products have a distinctive bite, chewiness, succulence, and firmness. These are called minced-type products as their texture is created by the size of the mince which can be fine, coarse, or flaky depending on the end-product. The texture can be further enhanced through tenderization (as in the case of *galouti kebab*), precooking involving boiling or steaming, and final cooking steps that can involve frying, tandooring, or grilling. These products have relatively more dry matter and mainly consist of a minced form of proteins and fats. On the other hand, whole-cut meat products or whole-muscle meat analogs, known as third-generation products, are the most technologically challenging products as they must structurally mimic the native form of meat. With other ingredients playing a minor role, plant protein quality and processing techniques are the major contributors to obtaining the fibrous layered structure as well as the juiciness that is a distinctive property of whole-cut meat. Typically, these products contain high water content up to 70%. Table 1 summarizes the role of various ingredients in a typical plant-based meat analog.

The main difference between the different types of meat analogs is that the first and second-generation products require binders, whereas the third-generation products do not need binders as the proteins are expected to form a continuous structure with networked fibers. In emulsion-type products, non-protein ingredients such as water, binders, salt, flavoring components, and coloring agents are present in relatively less proportion. Nevertheless, these minor ingredients influence the protein functionality and the product's organoleptic properties such as appearance, chewiness, texture, mouthfeel, juiciness, softness, and firmness. On the other hand, salt can change the structure of proteins as it impacts the ionic strength of the system and can toughen the end product (Pietsch et al. 2019). For all the above products, flavoring agents would be required to enhance and boost the taste and odor profile of the end products. The subsequent sections of this paper would focus on the third-generation or whole-muscle meat analogs.

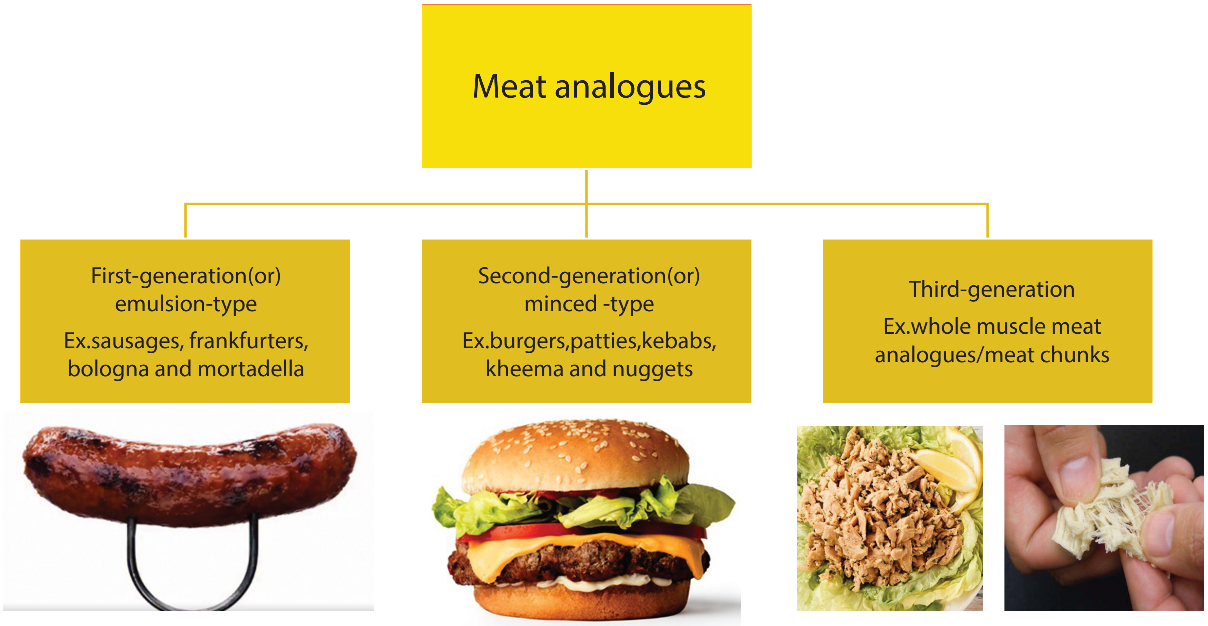

Figure 1. Classification of meat analogs
(Image sources: www.gfi.org)

Table 1. Ingredient and functionality mapping according to different types of meat analogs

Type of meat analog	Alternative names	Key ingredients	Main functionality required	Functionality mapped to ingredients
Emulsion type	First-generation	Water, salt, proteins, fats, carbohydrates (gums, fibers, starch),flavor	Emulsification, gelation, water Binding	Proteins: emulsification Binders: texture, gelation, stability Fats: juiciness
Minced-type	Second-generation	Water, proteins, fats, flavors, binders	Texture: chewiness, firmness, juiciness	Proteins: texture, water binding, fat binding Fat: juiciness Binders: hydration, binding,
Whole-cut meat	Third-generation Whole-muscle	Proteins, water, fats, hydrocolloids	Texture: water binding, juiciness, bite,	Proteins: texture, water-holding, fat binding Water & Fats: juiciness Hydrocolloids: water holding Fats can be added during or after the structuring process depending on the sensitivity of lipids and oils to thermal and shear stress

Overview of techniques for creating whole-cut/whole-muscle meat analogs

To create structured whole-cut meat analogs, two approaches have been explored. The first approach, referred to as the bottom-up approach, involves replicating the microstructure of animal meat by assembling individual elements to create a hierarchical architecture through techniques such as electrospinning, wet spinning, tissue engineering (for cultivated meat), and 3D printing. The second approach involves a top-down approach that aims at mimicking the material properties by transforming plant proteins through the application of thermal, chemical, and mechanical techniques. The result of these modifications is that proteins align themselves to create an anisotropic structure with fibrous layers; techniques such as high moisture extrusion, shear cell technology, and freeze-drying fall under this category (Dekkers et al. 2018). It should be noted that extrusion is the most mature technology among all the other top-down approaches. It has been explored extensively for creating TVPs, which are critical for preparing meat analogs. For those looking to set up a meat analog manufacturing facility, extrusion is the most accessible technology at the moment. Most other technologies are either still at the lab or pilot scale level or are governed by the patented intellectual property of companies working on commercialization of the technology.

Bottom-up approaches

Tissue engineering: Cultivated meat involves the use of tissue engineering techniques to create genuine animal meat derived through the cultivation and expansion of animal-derived muscle cells (Langelaan et al. 2010; Post 2012). By mimicking animal muscle tissue, cultivated meat replicates the sensory and nutritional profile of conventionally produced meat that is safe for consumption. The preliminary step involves identifying the animal species to extract muscle cells or stem cells and derive cell lines through a cell starter culture. This is followed by bench-scale optimization and co-culturing with adipocytes and fibroblasts to achieve a consistent output of muscle cells with or without support cells. In this process, standardizing various aspects of cell lines such as immortalization, proliferation, and differentiation, and ensuring the preservation of the cell lineage are critical aspects to be addressed. The next step involves pilot-scale reiterations that require novel bioreactor systems, media optimization and recycling, and sensors for automating the key steps of the process. Finally, industrial-scale production can be carried out through larger-scale bioprocessing units with scaffolding systems for cultivated meat production that can provide the best possible structure, texture, and taste - cost-effectively. A life cycle assessment of cultivated meat was recently published by CE Delft (co-commissioned by GFI and GAIA), which indicates that cultivated meat outperforms all conventional meat production in resource utilization, expressed as the feed conversion ratio (FCR) (Swartz 2021). FCR is a measure of livestock production efficiency, calculated as the ratio of the weight of feed intake to the weight gained by the animal. A lower feed conversion ratio indicates higher efficiency.

Fermentation of filamentous fungi: While filamentous fungi produce a wide range of products like antibiotics, organic acids, enzymes, and heterologous proteins, the most commonly known food application of filamentous fungi is the protein-rich product, Quorn, marketed by Marlow Foods. The key advantage of using filamentous fungi is the option to utilize the whole biomass for creating a meat analog. Additionally, filamentous fungi do not rely on traditional texturization technology like extruders, as the mycoprotein fibers closely resemble the short-range fibrous structures in minced meat products. Hence, these require only traditional unit operations for end-product creation. While the technology for mycoprotein production has existed for many years, companies have only recently started actively exploring large-scale production as new approaches to lowering capital and production costs. Consequently, approaches for making the processes efficient with higher biomass productivity have emerged.

Wet spinning: Wet spinning involves the formation of stretched filaments in the order of magnitude of 20 µm as protein precipitates and/or cross-links when immersed into a solvent system (Boyer 1954; Rampon et al. 1999; Tolstoguzov 1987). The protein added to the solvent system is in the form of an extrudate that is formed when a protein solution is forced through a spinneret. The precipitation, crosslinking, and solidification of the dispersed phase (extruded protein) occurs, and the continuous phase (solvent) is washed away to extract the fibrous structures. Production of food-grade fibers from plant-based materials like soy, pea, and faba bean has been reported in the literature (Gallant, Bouchet, and Culioli 1984; Rampon et al. 1999).

Electrospinning: Electrospinning involves growing thin fibers, in the order of 100 nm, from a biopolymer solution that is pushed to the spinneret. The electric potential of the spinneret relative to ground electrodes leads to the accumulation of charge on the surface of the biopolymer droplet at the tip of the spinneret, causing instabilities and subsequent fiber formations (Schiffman and Schauer 2008). Food-grade electrospinning creates nanofibers for carrying or delivering bioactive compounds like polyphenols and probiotics (Librán, Castro, and Lagaron 2017). Electrospinning using proteins such as whey, collagen, egg, gelatin, soy protein isolate, and zein finds applications in the encapsulation of bioactive compounds (Anu Bhushani and Anandharamakrishnan 2014; Ghorani and Tucker 2015). But, the globular nature of most native plant proteins and the insolubility of denatured proteins limit their utilization in electrospinning. It is desirable to use proteins that are highly soluble and have random coil structures.

Three-dimensional (3D) printing: 3D printing is an additive manufacturing technique that involves assembling or printing using a suitable food-grade solution or ink coming out from a fine syringe nozzle that moves layer by layer to create a meat analog (Dick, Bhandari, and Prakash 2019). A pre-designed digital model guides the printing process. The choice of materials used to create the ink, the physical and chemical properties of the ink, nozzle speed, size and specifications, and the digital model play key roles in determining the quality of the end product (Godoi et al. 2016; Nachal et al. 2019). 3D food printing is an innovative food structuring technique that facilitates the customization of the composition (proportion of ingredients), structure, texture, and taste of foods (Anandharamakrishnan and Ishwarya 2019). The potential of soy protein isolates for 3D printing has been explored in academic literature but not specifically for creating meat analogs (Chen et al. 2019; Phuhongsung, Zhang and Devahastin 2020). As 3D printing is an evolving field, research and development for applications in the alternative protein sector need to be furthered to make it a viable technological option in the future.

Top-down approaches

Protein-hydrocolloid solutions: This technique involves mixing proteins with hydrocolloids that precipitate in the presence of multivalent cations (Kweldam, Kweldam, and Kweldam 2011). Fibrous structures are formed as a result and are subsequently washed and pressed to remove excess water to obtain a product with 40-60% dry matter. The washing and pressing steps can destroy long-range order, thus limiting the application of this technique to minced meat analogs. This process has mainly been reported in a patent filed by Adriaan Cornelis Kweldam, Gretha Kweldam, and Arjen Cornelis Kweldam and should be explored further using a combination of plant protein sources like soy, rice, maize, lupine, etc. with various hydrocolloids. A dairy-based meat substitute called Valess, launched in 2005, based on a casein and alginate system, was created using this technique.

Freeze structuring: Freeze structuring involves the formation of anisotropic protein fibers by freezing a protein solution or slurry of proteins. When heat is removed unidirectionally without mixing, the alignment of ice crystal needles leads to the formation of short-range structures of proteins. The frozen protein fibers are dried without melting to obtain a porous microstructure with proteins parallelly oriented along the direction of the sheets that are connected to form a fibrous product (Consolacion and Jelen 1986). This technique has been used for structuring meat, fish, and plant proteins (Consolacion and Jelen 1986; Lugay and Kim 1978; Middendorf, Waggle, and Cornell 1975). The freezing temperature and rate of heat removal determine crystal needle size and subsequently the size of the protein fiber. To yield fibrous structures, the protein must be water-soluble and insoluble at freezing temperatures (Lugay and Kim 1978).

Shear cell technology: Shear cell technology utilizes a shear cell which is based on the design of rheometers and has a cone-in-cone or Couette geometry, which creates shear flow deformation leading to the formation of fibrous structures from proteins (Manski et al. 2007b; Manski et al. 2007a; Krintiras et al. 2014; Van den Einde et al. 2004). Shear cell technology has been used to create fibrous products from calcium caseinate, soy protein concentrate, soy protein isolate, and wheat gluten, and soy protein isolate and pectin (Dekkers, Nikiforidis et al. 2016; Grabowska et al. 2014; Grabowska et al. 2016; Manski et al. 2007b). The fibrous structures created using plant proteins have shown anisotropy up to the micrometer scale. Krintiras et al. (2016) demonstrated pilot-scale trials with a shear cell device having a 7L capacity for producing soy-based meat analogs.

Mechanical elongation: Mechanical elongation involves using a mix of protein isolates or concentrates and subjecting them to mechanical processing in a food processor to stretch and pull which leads to protein agglomeration to form a dough with strong networks. This dough is further stretched and elongated using a noodle maker and folded to form the final dough. The dough is steamed in a food steamer and subsequently cooled overnight to obtain the end product. Mechanical elongation is a relatively new approach. Research on the applications of this technique for creating meat analogs is at its nascent stages. A notable paper recently published by Chiang et al. (2021) explored the usage of varying proportions of wheat gluten and soy protein isolate using mechanical elongation to create meat analogs.

Extrusion: Extrusion or extrusion cooking is an established commercial process for the manufacturing of proteinaceous and fibrous products from plant-based proteins. It entails a combination of physical and chemical changes, during which the raw materials are subjected to high temperature (heating), high pressure, hydration, and strong frictional and shear forces, whilst they are mixed uniformly within the barrel of an extruder. Liquid ingredients like water and semi-solid components such as fats are added inside the extruder and mixed by the action of rotating screw elements. As the material flows through the extruder with the combination of shear and temperature profile in the barrel, it undergoes a cooking process (e.g., gelatinization of starch and denaturation of proteins) while the pressure builds up in the system. Given the relatively short residence time (<1 minute) of the material in the barrel, the process is also called high-temperature short time or extrusion cooking. If there is no cooling tunnel at the end of the barrel, the material expands as a result of a sudden pressure drop, allowing the evaporation of water and the formation of air bubbles in the solid matrix. The steam thus formed leads to an expanded product with a lower water content that may potentially fragment into small pieces (Harper, 1989; Högg et al., 2017). This yields a puffed product if starch is used and a spongy texturized vegetable protein if proteins are used. If there is a cooling die at the end of the barrel, the expansion is curtailed and the material solidifies with closely packed layers and fiber formation when proteins are used as feed material. The material that exits the die undergoes hot cutting and is dispatched for downstream processing which can include drying, frying, seasoning, and packaging depending on the end application.

Depending on the feed moisture content, extrusion technology can be classified into low-moisture extrusion (LME; feed moisture content: 10%–40% [w/w]) and high-moisture extrusion (HME; feed moisture content: >50% to 70% [w/w]). LME is the preferred manufacturing method to produce dry-textured vegetable proteins (TVPs), which are expanded products that must be rehydrated before consumption (Malav et al. 2015). Contrastingly, HME technology is used to produce moist and fibrous products such as whole-muscle meat analogs. A feed moisture content above 40% is necessary to create an anisotropic structure that mimics whole-muscle meat (Schmid et al. 2022). An anisotropic structure is signified by a preferred orientation of dimensional characteristics in the structure (Hilliard 1967).

Low-moisture extrusion (LME): This method typically uses starch and protein as feed ingredients. The moisture content in this process is less than 30% on a weight-by-weight basis and is characterized by a lack of cooling tunnel/die at the end of the barrel. The material obtained is an expanded or puffed product. While single-screw extruders were the first to be used for low-moisture extrusion cooking, twin-screw extruders are now most commonly used due to several advantages associated with energy efficiency and versatility. Low-moisture extrusion has been widely used to prepare texturized vegetable proteins that are hydrated, and further used to create various types of plant-based meat analogs, especially for applications in minced meat analogs and emulsion-type meat analogs.

High-moisture extrusion (HME): HME is a continuous cook-and-stir process that results in a product that replicates the appearance and fibrous texture of conventional whole-muscle meat. The moisture content in this process is in the range of 40% to 70% on a weight-by-weight basis. This method typically uses plant-based proteins as feed ingredients to create fibrous and layered structures. Twin-screw extruders are used for high-moisture extrusion processes. The working principle of a twin-screw extruder used for HME is shown in Fig. 2. The rotation of the screw inside the barrel is responsible for mixing and conveying. The friction experienced by the material inside the barrel when in contact with the surface of the screw and the barrel walls is responsible for shearing as well as an increase in the temperature due to viscous dissipation. As the material moves through the extruder, changes in chemical and physical properties of the material, and the orientation of protein chains along the direction of shearing, lead to the formation of fibrous structures. The presence of a long cooling die at the end of the heating zone facilitates controlled cooling after melt formation. Cooling prevents the water-to-steam conversion in the product as it exits the die. Consequently, water is retained in the fibrous structure making the end product similar to that of whole-cut meats. Currently, the HME technology accounts for about 20% of global plant-based meat products and is expected to grow in double digits by 2030, as more manufacturers worldwide adopt this process (New Food 2022).

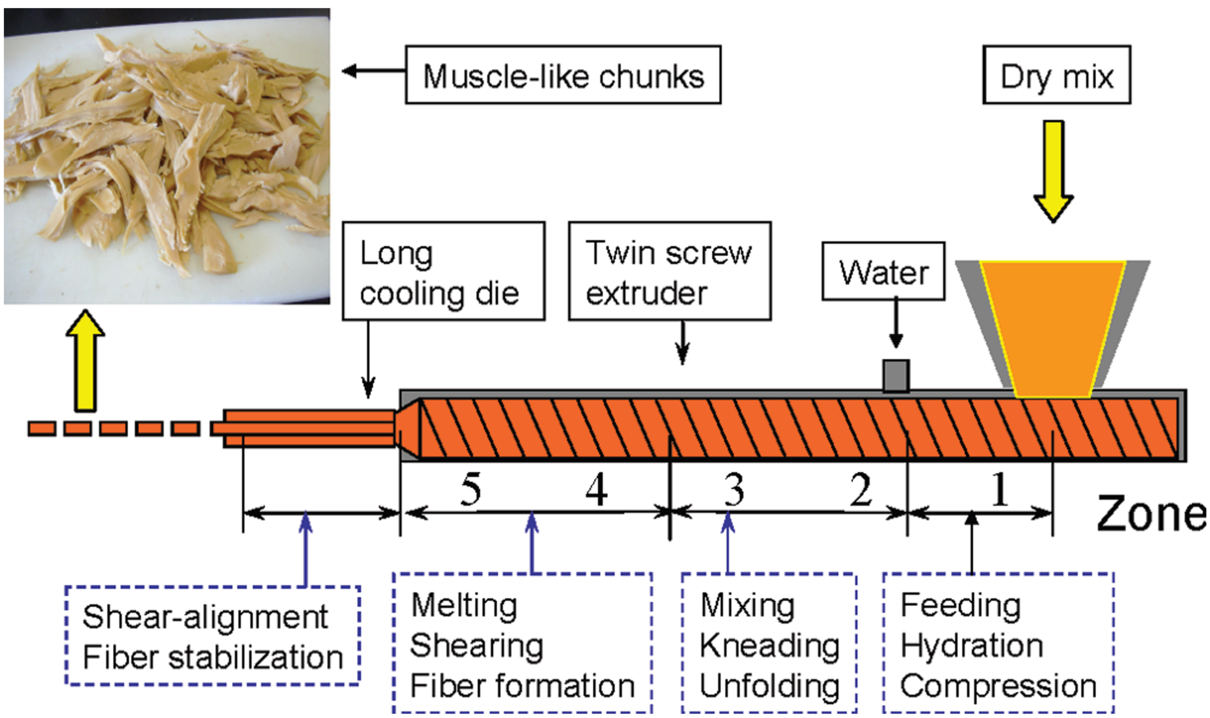

Figure 2. Working of a twin-screw extruder for high-moisture extrusion of proteins to form fibrous meat analogs

(Source: Liu and Hsieh 2008)

A deep dive into the high-moisture extrusion technology for the development of whole-cut meat analogs can be found in the upcoming sections.

Raw material for high-moisture extrusion

Animal proteins versus plant proteins

It is critical to understand the structure of animal meat before discussing the technological aspects of creating plant-based meat using high-moisture extrusion. Meat is composed of water, proteins, minerals, fats, vitamins, minor quantities of carbohydrates, and other bioactive components. Meat may also contain nerve tissue, blood vessels, cartilage, tendons, bone, skin, and organ tissue. On a dry basis, protein is the main component of meat, followed by fat, with much of the protein present in the skeletal muscles of animal species. Depending on the animal species and muscle type, a typical muscle fiber is 1-40 mm long and 20-100 µm in diameter. The muscle fiber is composed of myofibrils, which comprises myofilaments. Myofilaments consist of elongated fibrous cells arranged in a complex hierarchical architecture with myosin and actin as the main structural components, as shown in Fig. 3 (Bioactive Collagen Peptides 2023).

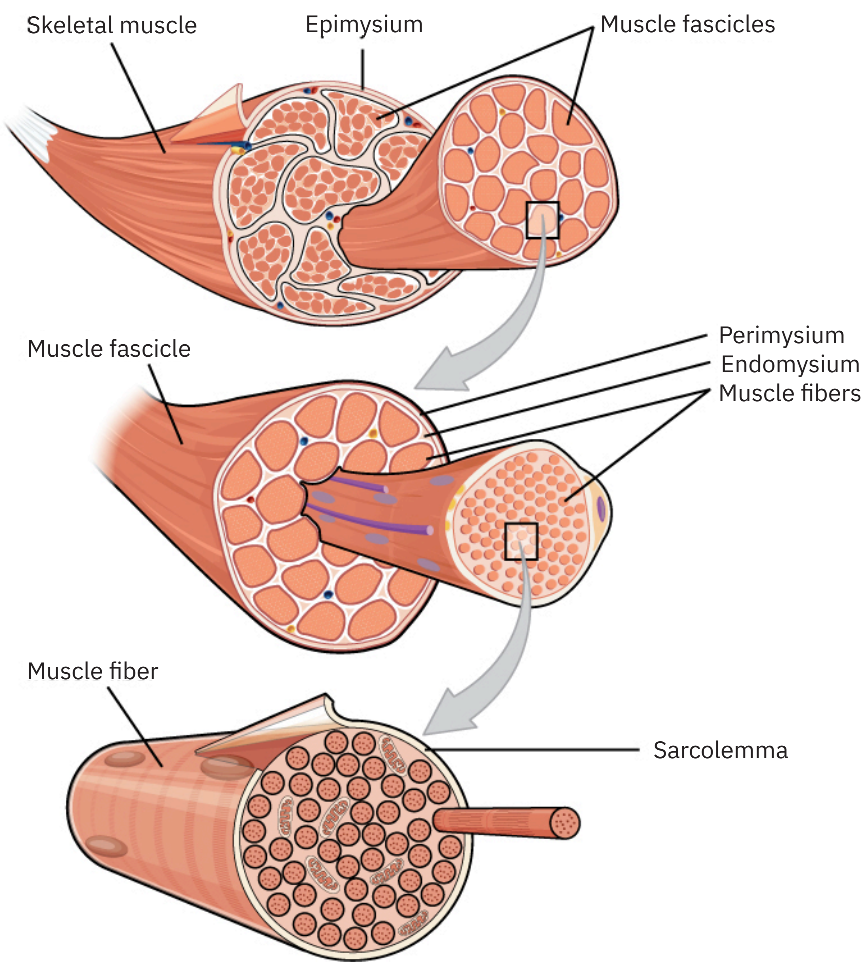

Figure 3. Structure of animal muscle fiber

The interstitial spaces in myofilaments facilitate the inclusion of water through hydrogen bonding and dipole interactions. The texture and juiciness in whole-muscle meat can be attributed to the unique cellular arrangement of fibrous protein along with collagen fibrils surrounding the muscle bundle and water distribution within the myofilament matrix in the muscle fiber. The interstitial spaces in the muscle fiber matrix entrap about 85% of the water found in fresh meat (Puolanne and Halonen 2010) due to the capillary effect in the interstices, allowing the retention of water during cooking and the eventual release of water during mastication (chewing), providing a juicy mouthfeel. The emulsification capacity of muscle protein is due to the unique fibrous structure arising from the aspect ratio (length-to-diameter ratio) of fibers and polar structure (Sha and Xiong 2020). The inherent techno-functional properties like emulsification capacity, water holding capacity, gelation, and oil binding capacity of muscle proteins play a critical role in creating the chewiness, tenderness, firmness, and mouthfeel found in processed meat products like patties, sausage, and bologna.

On the other hand, proteins found in terrestrial plants are storage and metabolic proteins that provide the nutrients needed for plant growth and development (Herman and Larkins 1999; Shewry 2003). This difference in protein types can be attributed to the fact that polysaccharides provide structural properties and movement in plants that is facilitated by changes in turgor pressure. Thus, plants do not require muscle contraction, unlike animals which rely on muscle protein contractions to enable movement (Cosgrove et al. 1984; Morillon et al. 2001).

Plant proteins found in nuts, legumes, oilseeds, and cereals are storage proteins. As storage proteins, plant proteins in their native globular structure are typically incapable of forming fibrous structures found in animal muscles. Hence, the proteins need to be structured in a way to replicate the texture of animal protein. It is important to note here that creating fibrous structures similar to animal muscle at a microscopic level would require assembling proteins and non-protein components, which can be accomplished by processes like micro-scale 3D printing, electrospinning, wet spinning, or by developing cultivated meat. The inherent complexity of creating such an arrangement at a microscopic level would likely be reflected in the unit cost of creating such products and would require substantial technical innovation and scale-up efforts to attain price parity with animal meat. On the other hand, extrusion-based structuring technology relies on creating a macroscopic structure. It is implicit that shear-based structuring mechanisms such as extrusion are not aimed at microscopic arrangement but creating long-range order which eventually provides the same texture and resembles animal muscle on attributes like chewiness, tenderness, firmness, juiciness, and mouthfeel (Dekkers et al. 2018). Further, post-processing of extrudates to enhance flavor and incorporate fat components is required to replicate the sensory experience of eating meat.

An understanding of the protein type, composition of plant protein source, and structural properties of proteins is key to a smart selection of ingredients for creating meat analogs. Experimental data on various protein isolates and concentrates used to prepare high-moisture meat analogs (HMMA) is crucial to understand the correlation between the composition of proteins and end-product properties. Such correlations can enable product developers to try new blends and explore a wide range of functional properties in HMMA. Thus, the subsequent sections of this report would present a comprehensive discussion of the classification, sources, and characteristics of plant proteins.

Types of plant proteins

Proteins can be classified based on different parameters such as solubility, function, location, and homology. The solubility-based classification is popular because of its simplicity and its relevance from a protein extraction perspective. Osborne (1907) grouped proteins into 4 classes based on their solubility in water (albumins), salt water (globulins), alcohol (prolamins), or acidic water (glutelins). Further knowledge about the proteins such as their size, sedimentation rates, and homology has allowed us to further classify the proteins into subgroups. These subgroups help us understand the functionality of the proteins better.

Albumins: Albumins are water-soluble proteins and consist of enzymatic proteins, lectins, protease inhibitors, and amylase inhibitors. Albumins typically have a molecular weight ranging from 10,000 to 80,000 Da (Grossman and Weiss 2021; Boye, Zare, and Pletch 2010) and a 2S sedimentation coefficient. Sedimentation coefficients indicate the rate of sedimentation of a macromolecule which is a function of particle size, shape, and molecular weight. The higher the molecular weight and density, and the more the spherical nature of molecules, the higher the sedimentation rate and thus, the higher the sedimentation coefficient. Albumins demonstrate a higher solubility than globulins over a broad pH range because of lower molecular weight and higher hydrophilicity. The solubility of albumins derived from kidney beans, peas, chia, hemp seeds, buckwheat seeds, locust beans, pumpkin seeds, and sunflower seeds at different pH values have been reported in the literature (Djemaoune et al. 2019; González-Pérez et al. 2005; Julio et al. 2019; Lawal et al. 2005; Malomo and Aluko 2015; Mundi and Aluko 2012; Pham et al. 2017). The versatile solubility profile of albumins makes them relevant for alternative protein applications, especially for products that require soluble protein components like plant-based dairy and egg products.

Globulins: Globulins are salt-soluble proteins and are classified into three protein categories - 7S, 11S, and 15S - based on their sedimentation coefficient. Of the three types of globulins, 7S and 11S are the most commonly found. 7S globulins have a molecular weight in the range of 150,000 to 190,000 Da and a trimeric structure without disulfide bonds and exhibit glycosylation. 11S globulins have a molecular weight between 300,000 and 370,000 Da and have a hexameric structure with disulfide bridges (González-Pérez & Arel-lano 2009). The solubility characteristics of globulins are largely dependent on the ionic strength and pH of the solution. Resulting from differences in the type of proteins, intrinsic factors such as molecular weight, chemical and charge properties of subunits, and dissociation and association of subunits also impact solubility (Gueguen et al. 1988; Kimura et al. 2008; Lakemond et al. 2000; Molina et al. 2004). Lower molecular weight favors solubility while high ionic strength and neutral pH favor the native state of globulins (Gueguen et al. 1988; Kimura et al. 2008; Withana-Gamage et al. 2015).

Prolamins and Glutelins: Prolamins are storage proteins that are insoluble in water or salt solutions in their native state but are soluble in alcohol and water mixtures either in their native state or after the reduction of disulfide bonds. Prolamins mainly consist of proline and glutamine; these are usually found in cereals. Glutelin proteins found in some cereal proteins are often described as a separate class of proteins. However, under the broader definition of solubility of prolamins, glutenins can be categorized as a subset of prolamins due to their solubility in the water/alcohol system upon reduction of disulfide bonds. Gluten is one of the key components of wheat protein. Wheat gluten consists of gliadin and glutenin (a type of glutelin). Gliadin is alcohol soluble and has polypeptides linked by intramolecular disulfide bonds. These have a lower molecular weight due to their monomeric nature and are structurally similar to low molecular-weight glutelin. Glutelin is soluble in dilute acid or alkali detergents and chaotropic or reducing agents (Osborne, 1924) and is insoluble in water at a neutral pH. Glutelins have a higher proportion of methionine and cystine, making them nutritionally relevant. These have polymeric aggregates of high molecular mass and low molecular mass subunits held together by disulfide bonds. The strength and elasticity of wheat dough comes from glutenin. These proteins have the potential to form intramolecular and intermolecular cross-links through disulfide bonds at high temperatures; disulfide bonds can be intramolecular or intermolecular, depending on the protein class.

The uniqueness of albumins, globulins, glutelins, and prolamins with respect to their role as ingredients in whole muscle meat analogs is their ability to form anisotropic or fibrous structures. While hydration facilitates the self-assembly of glutelins and prolamins to form a three-dimensional network, albumins, and globulins demand physical, chemical, or enzymatic modifications to achieve the desired anisotropicity in plant-based whole-muscle meat analogs (Sim et al., 2021). The content and relative proportions of these plant protein types vary with their sources, which are discussed in the forthcoming section.

Plant protein sources

We can classify key plant protein sources as cereals/grains and pseudocereals, legumes and pulses, oilseeds and edible seeds, and tubers (Fig. 4).

Cereals and Pseudocereals: Cereals and grains are terms used interchangeably and refer to grasses of the Poaceae family. Examples of cereals include rice, wheat, maize, millet, rye, barley, and sorghum. Another category called pseudo-grains or pseudocereals includes buckwheat, chia seeds, quinoa, and amaranth. Cereals can be consumed as seeds (rice, oats, barley, and maize), flour (wheat, maize, and rye), or flakes (barley, maize, and oats); these are rich in carbohydrates, oils, and fats with relatively lower protein content compared to legumes and oilseeds. For example, wheat has 8% to 17.5% protein; maize has 8.8% to 11.9% protein; barley has 7% to 14.6% protein; rice has 7% to 10% protein; oats have 8.7% to 16% protein; and rye has 7% to 14% protein (Guerrieri 2004). The proteins found in cereals are generally prolamins and glutelins (Delcour et al. 2012; Shewry and Halford 2002; Veraverbeke and Delcour 2002). On the other hand, pseudocereal proteins are rich in a combination of albumins and globulins (Janssen et al. 2017; Klose and Arendt 2012). Wheat gluten is a commonly extracted product from the recovery of wheat starch in the wet processing of wheat flour (Wang et al. 2006; Xiong, Agyare, and Addo 2008). The ability of wheat gluten to form blends with viscoelastic properties plays an important role in the structuring process.

Legumes: Legumes are plants of the Fabaceae family characterized by their ability to fix atmospheric nitrogen and typically contain 20 to 30% protein by dry weight (Riascos et al. 2010). Legumes include beans, soybeans, peas, chickpeas, peanuts, lentils, and lupins. Legumes account for 25% of primary crop production. Pulses are dried seeds of leguminous plants and are rich in proteins. Examples of pulses include dry beans, dry broad beans, dry peas, chickpeas, pigeon peas, lentils, and lupins. Proteins found in pulses are usually globulins and albumins. The major storage proteins in legumes are globulins and albumins with globulins in higher proportion. Globular proteins with 7S and 11S sedimentation coefficients have been reported. 7S are referred to as vicilin and are trimeric with molecular weight in the range of 150 to 170 kDa (Plietz et al. 1983). Vicilins lack cysteines and have no disulfide bonds (Casey, Domoney, and Ellis 1986). 11S are referred to as legumin and are oligomers with disulfide linkages. The exact composition of the proteins depends on season and variety (Boye et al. 2010). An in-depth understanding of the composition, nutritional profile, and functionality of pulse proteins can be obtained from several literature reviews by Boye et al. (2010), Fukushima (1991), Lam et al. (2018), Singh et al. (2015).

Oilseeds: Oilseeds consist of crops that are grown for oil extraction. Examples of oilseeds include sunflower, sesame, rapeseed, mustard, flax, cotton, and canola, to name a few. Proteins can be extracted from oil meals left after oil extraction. The protein content in oilseeds varies from 10% to 40% depending on the species. For example, safflower, cottonseed, and rapeseed contain 13-17% (Prakash and Rao 1986), 23% (Sunilkumar et al. 2006), and approximately 25% (Serraino and Thompson 1984) of protein, respectively. Oilseeds contain all four types of proteins - albumin, prolamin, glutenin, and globulin - in varying proportions (Prakash and Rao 1986; Minakova et al. 1996; Marcone 1999). The major proteins found in oilseeds like rapeseed and sunflower include 11S globulins. However, glutelin or albumin can be the main fraction in some seeds like pumpkin, depending on the species (Campbell et al. 2016; González-Pérez and Vereijken 2007; Pham et al. 2017). The similarity between the 11S proteins in oilseeds and legumes has been reported (Grinberg et al. 1989; Lampart-Szczapa 2001) with similar molecular weights, amino acid profiles, subunits, and secondary structure (Marcone 1999). However, differences in the tertiary structure have been reported (Marcone 1999).

Soybean is a seed of a leguminous plant but is classified as oilseed due to its high oil content. It also has the highest protein content (about 36%) among all plant protein sources. The major component of soy proteins is globulin which includes β-conglycinin and glycinin. β-conglycinin is trimeric with a 7S sedimentation coefficient and molecular weight between 150 and 200 kDa (Mitsuda and Kusano 1965) and glycinin is hexameric with an 11S sedimentation coefficient and molecular weight in the range of 300 to 380 kDa (Staswick et al. 1984; Sun et al. 2008).

Tubers: Another class of protein called patatin is found in tubers like potatoes. These have a dimeric structure and low molecular weight. Table 2 summarizes the reported composition of various plant protein sources.

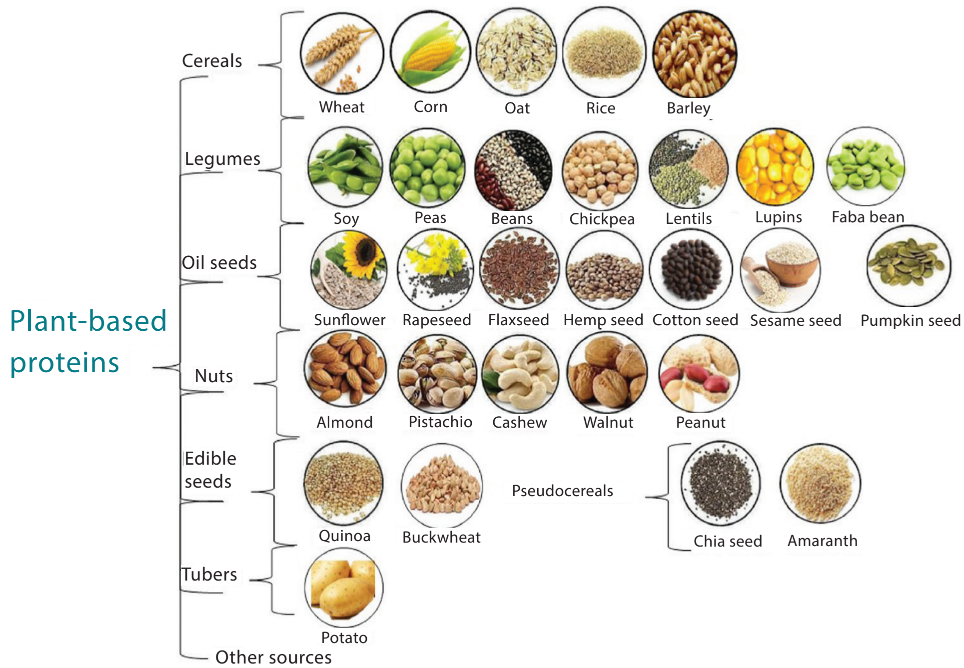

Figure 4. Plant protein sources

(Source: Nikbakht Nasrabadi et al. 2021, CC BY 4.0.)

Table 2. Composition and protein quality of various plant sources
(adapted from Grossmann and Weiss 2021)

Type of crop	Composition	Total protein (%)	Globulin (%)	Glutelin (%)	Prolamin (%)	Albumin (%)	Limiting amino acid	Protein Quality (DIAAS(D)/ PDCAAS(P))
Cereal	Rice	7-9	12	80	4	2-6	Lys + Thr	59.5 (cooked rice) (D)
	Wheat flour	8-15	5-8	40	35-40	6-10	Lys	54 (D)
	Maize	9-12	4	26	60	4	Lys + Trp	28.5-67 (P)
Legumes and pulses	Pea	20-30	50-60			15-25	Met + Cys + Trp	46-73 (D)
	Soybean	35-40	90				Met + Cys	105 (flour, D)
	Lupin	35-40	42-51	10	10	18	Met + Cys	81 (P)
	Lentils	20.6-31.4	70	11	3	16	Met + Cys	49-58 (D)
	Chickpea	14.9-30.6	53-60	19-25	3-7	8-12	Trp	67 (D)
Oilseed	Canola	17-26	60	15-20	2-5	20	Lys	95 (isolate, D)
	Sunflower	20-40	56	17	1	22	Lys + Ile	67 (D)
	Pumpkin	24.5-36.0	20.4	49	4.3	13.5	Ile + Val	-
Tuber	Potato	22	9	23	1.4	67	Met or Ile	102 (isolate, D)

Studies have reported the use of soy protein concentrate, soy protein isolate, pea protein isolate, wheat gluten, peanut protein isolate, faba bean protein isolate, lupin protein isolate, soy protein fraction, soy flour, wheat starch, peanut protein concentrate, microalgae, and defatted peanut flour as ingredients to produce high-moisture meat analogs using either twin-screw extruders or shear cell technology. A compilation of these studies can be found in the table below.

Table 3. Overview of plant-based ingredients and combinations and process conditions used for creating meat analogs using extrusion technology (adapted and modified from Cornet et al. 2022).

Equipment used	Material	Moisture content (%)	Maximum temperature (ºc)	References
Twin-screw extruder	Soy protein isolate + Defatted soy flour	60%, 70%	150	Mateen, Mathpati and Singh (2023)
Twin-screw extruder	Soy protein concentrate + Microalgae	60	140	Caporgno et al. (2020)
Twin-screw extruder	Peanut protein	55	155	Zhang et al. (2020)
Twin-screw extruder	Soy protein concentrate + Wheat gluten	60	170	Chiang et al. (2019)
Twin-screw extruder	Lupin protein isolate + Microalgae	50	140-175	Palanisamy et al. (2019)
Twin-screw extruder	Soy protein concentrate	60	100, 140, 160	Pietsch et al. (2019)
Twin-screw extruder	Soy protein isolate + Wheat gluten + Corn starch	70	160	Samard, Gu, and Ryu (2019)
Twin-screw extruder	Pea protein isolate	60	140	Osen et al. (2015)
Twin-screw extruder	Soy protein isolate + Wheat gluten	50	148	Zhang et al. (2015)
Twin-screw extruder	Soy protein isolate	50	150	Fang, Zhang, and Wei (2014)
Twin-screw extruder	Pea protein isolate	55	100-160	Osen et al. (2014)
Twin-screw extruder	Soy protein isolate + Wheat gluten + Wheat starch	60-72	170	Liu and Hsieh (2008)
Single-screw extruder	Pea protein isolate+ Defatted peanut flour	50-55	165	Rehrah et al. (2009)

The underutilization of protein sources other than soy, pea, and wheat can be attributed to many factors. Some of the key factors include the availability or volume production of the crop and the per capita protein content, which impact the accessibility and cost of protein isolates or concentrates. Second, is the availability of infrastructure and scalable technologies for protein extraction after the oil fraction is extracted. The third is the availability of data on the functionality of protein concentrates/isolates and demonstrated use cases for creating end-products like meat analogs. A variety of plant-based proteins should be exploited to create products that cater to diverse textures and taste profiles attributed to different meat types (ex. chicken, pork, mutton, beef). Fourth is the underdeveloped technical capability to create 'neutral' protein concentrates and isolates that are devoid of flavor, fat, and polyphenols. For example, most chickpea proteins exhibit a strong taste. As plant-based meat products evolve and consumers expect additional nutritional benefits, utilizing ingredient blends from various crop sources would enable meeting not only essential amino acid requirements but also fiber, vitamin, and mineral needs. Low-cost and neutral-tasting protein sources with complete nutritional profiles, no allergenicity, and multiple high-volume sourcing options will become the mainstay. Additional details on the bottlenecks for crop optimization and ingredient processing can be found on GFI's Science of Plant-Based Meat webpage.

For a detailed comparison of various plant proteins on digestibility, nutritional value, amino acid profile, and allergenicity, readers are encouraged to explore the work of Asgar et al. (2018). Readers can also refer to The Plant Protein Primer targeted at providing manufacturers to help compare plant protein sources on key attributes like nutrition, functionality, price, and sourcing. The guide includes profiles of major and emerging plant protein sources, combination and processing strategies, and consumer perceptions. It is to be noted that data on price and sourcing is specific to the US market.

Mapping functionality to ingredient properties

Of the variety of functionalities offered by varied ingredient sources and their components relevant for creating meat analogs, the properties most essential for creating fibers and anisotropic structures in whole-cut meat analogs are yet to be determined. Detailed information on the functionality of different protein sources can be found in the report by Kelba and Ismail.

The following properties are of relevance for creating whole-cut meat analogs:

1. Fibration capacity(technical parameter): Fibration capacity is defined as the tendency to form a fibrous structure under a standard range of processing conditions. The fibration capacity indicates the potential of a protein source for utilization in creating meat analogs. At a bench scale, fibration can be observed by processing the material in shear cells, capillary and closed cavity rheometers, or through mechanical elongation techniques. Creating a quantitative parameter such as fibration capacity will be beneficial for the alternative protein sector as it will help benchmark ingredients against standard ingredients like soy protein isolate, wheat, gluten, or pea protein isolate and help customers make an informed ingredient selection. It can be related to the degree of anisotropy observed in the extrudate, which can be measured by differences in cutting strengths along perpendicular directions.
2. Water holding capacity(technical parameter): Since water content in HMMA is in the range of 50-70%, the water holding capacity of plant proteins is of key importance in producing HMMA. Low water-holding capacity would prevent the structuring of high water content and, subsequently, prevent the formation of a coherent solid extrudate.

3. Emulsification(technical parameter): To ensure HMMA can hold fat during and after extrusion, good emulsification capacities of proteins as well as carbohydrates or hydrocolloids present in the ingredient mix are necessary to encapsulate and stabilize fats.
4. Other properties(technical parameter): Viscoelasticity, gelation capacity, and solubility are the other relevant functionalities for preparing HMMA. However, robust data on the relationship between these parameters and end-product properties needs to be established.
5. Juiciness(sensory parameter): The mouthfeel attributed to whole-cut meat analogs is a combination of texture and juiciness. Juiciness can be influenced by the amount of water and fat inclusion and their release characteristics during mastication. A study by Wi et al. (2020) found that water contributes more than fat toward juiciness. While the textural properties like chewiness and hardness have been addressed to some extent through fiber formation during the extrusion process, due to the microstructural differences in animal muscle fiber structure and plant-based HMMA, the sensory effect produced by capillary systems in animal muscle responsible for juiciness remains to be explored for HMMA.

Components of a twin-screw extruder for high-moisture extrusion

Extruders can be viewed as a combination of three components in a series which includes the feeding zone, screw section (conveying zone, mixing zone, melting zone), and die (Fig. 2).

Feeding zone: In the feeding zone, the barrel is maintained at room temperature, and the screw has conveying elements for delivering the material to the next zone with little shearing (Zhang et al. 2015). Dry ingredients are added at this stage using a gravimetric or volumetric feeder; gravimetric feeding systems are most suitable for obtaining well-controlled and evenly distributed feed for consistent extrusion.

Screw section: The material then moves to the conveying zone with converting elements that allow the material to move forward. Depending on the requirement, short-pitch conveying elements can be used to increase compression besides facilitating the volumetric transfer. After this, the material moves to the mixing zone which has kneading elements that mix dry feed material with incoming liquid components. For uniform water injection, it needs to be injected at less than 80°C. The mixed material then moves to the melting zone with more kneading elements than the previous zones. The material is subjected to high temperatures (above 130°C) and the shearing action in this zone modifies the physical and chemical properties of the protein (Zhang, Liu, et al. 2017; Thiebaud, Dumay, and Cheftel 1996). Denaturing of protein occurs in this region as chains unfold in the direction of the flow, exposing hydrophobic amino acids (Akdogan 1999; Day and Swanson 2013). Protein-protein and protein-water interactions determine modification in protein conformation in this region (Zhang, Ying, et al. 2017; Manoi 2009; Pietsch, Emin, and Schuchmann 2017). Depending on the requirements of the process, reverse elements may be used to increase residence time and mixing, and rupture blocks can be used for enhanced backflow and mixing.

Die section: The material then moves to the die or tunnel which can be short or long depending on the desired product. The die head at the end of the tunnel provides shape to the material that exits the extruder system and helps build pressure in the system. For low moisture extrusion, the die is short, and the material emerges without cooling and experiences a high-pressure drop. For high moisture extrusion, the cooling tunnel is long, and the temperature is maintained well below 75°C to prevent evaporation. The drop in temperature and pressure, and the presence of shear stress perpendicular to the direction of extrusion in the die may lead to phase separation and laminar flow which contribute to the formation of a fibrous structure and thereby aid in the structuring process (Thiebaud, Dumay, and Cheftel 1996; Osen et al. 2014; Maurya and Said 2014).

Ancillary components: The extruder system requires a set of tools and equipment before, during, and after extrusion cooking to help the process run smoothly. These include a feeder, a hot and cold water circulation system for heating the barrel and cooling the die, a peristaltic pump vacuum system, pressure transducers, thermocouples, a cutting device, a unit to control the feeder and cutting device, and cutting blades. Various cleaning and maintenance tools are also required.

The transformation of feed material to fibrous and anisotropic structures in the extruder results from a combination of thermal and mechanical energy inputs. Thermal energy supplied to the system is a function of the barrel temperature and steam injected. On the other hand, mechanical energy input is a function of power required to move and rotate the screw which manifests in the form of conveying, mixing, shearing, and pressure buildup. The ratio of specific thermal and mechanical energy inputs in combination with moisture content determines the type of end-product formed. While only thermal energy is responsible for the gelatinization of protein mass, the input of mechanical energy leads to pellet formation or expansion of the product depending on moisture content. High moisture extrusion leads to the formation of products that are intermediate between the above two.

Parameters of the high-moisture extrusion process

High-moisture extrusion is a multivariate process that depends on process or design parameters such as screw configuration, length-to-diameter ratio of the screw, and die geometry besides the system and product parameters (Fig. 5). Process parameters are the ones that can be controlled by a user. Change in merely one design aspect or process parameter can impact other process conditions. For example, an increase in screw speed can impact temperature profile, and pressure inside the barrel and at the cooling die. In a twin-screw extruder, screw elements can be customized depending on the requirements and be used to create specific sections with elements for conveying, kneading, rupture, mixing, reverse mixing, and so on. As mentioned before, a combination of thermo-mechanical processing in extruders allows for multiple unit operations such as mixing, kneading, shearing, heating, cooling, shaping, and forming. Raw material properties as influenced by their source and composition of proteins have already been discussed in the earlier sections of the report. The impact of processing and system parameters and their correlation with the product parameters have been discussed in the section below.

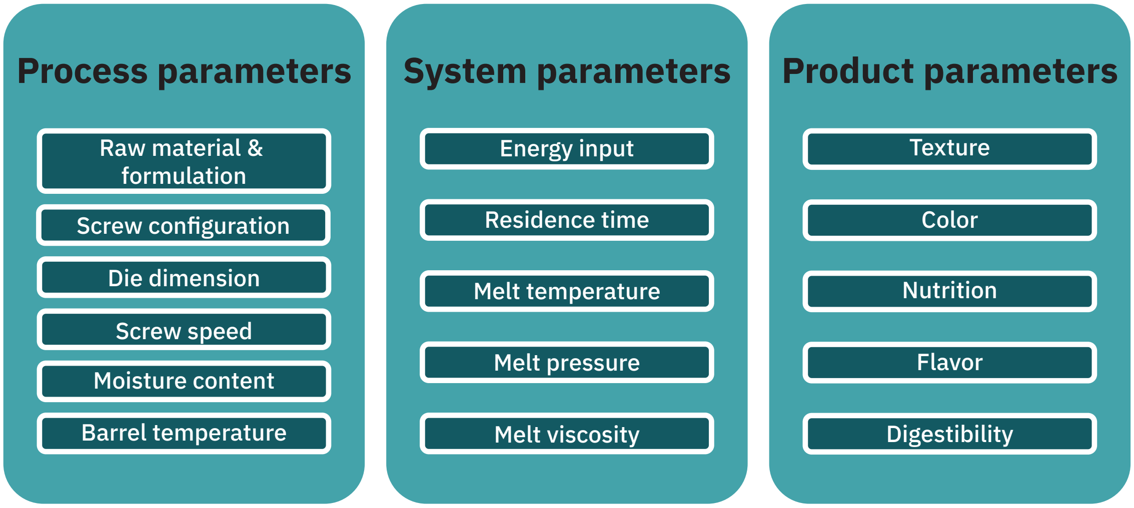

Figure 5. Parameters of extrusion

It is to be noted that the system parameters such as residence time, temperature and pressure profile, and rheological properties manifest as the result of a combination of the process parameters. Quantifying the degree of contribution of each parameter to changes in process and product parameters has been challenging but should be explored to develop computational modeling techniques that can one day enable the prediction of recipes and process conditions that can yield the desired product attributes. Some of the important parameters from a design perspective include the following:

a. L/D: the ratio between the length and diameter of the barrel; L/D ratios reported in the literature are in the range of 15:1 to 30:1; higher L/D ratios allow for higher throughput and higher residence time, given all other parameters are constant
b. Do/Di: the ratio between the outer and inner diameter of the screw; typically high diameter ratio allows for higher volumetric feeding and overall higher throughput
c. Screw speeds: the screw speed would depend on the motor capacity as well as the resilience of the system to withstand high shear and mechanical energy input

Impact of process parameters

Temperature: Temperature is one of the critical processing parameters responsible for the transformation of protein properties like protein-protein interactions, protein-water interactions, and flow behavior that contribute to the formation of anisotropic and fibrous structures (Grabowska et al. 2016; Krintiras et al. 2015; Pietsch et al. 2019). It also influences conformational changes leading to unfolding, alignment, collision, and aggregation through modifications in the covalent and non-covalent interactions (Fang et al. 2013, 2014; Osen et al. 2015; Wolz and Kulozik 2015; Zhang et al. 2019). Generally, in the high-temperature range where fibrous structure formation is feasible, temperature enables a reduction in the microstructural interface between various phases leading to stronger adhesion and interaction effects, inducing higher tensile strength in the end-product (Dekkers et al. 2016; Dekkers et al. 2018). High temperatures can also lead to degradation reactions, leading to a change in color, as with Maillard reactions due to the interaction of amino groups with reducing sugars. High temperatures can also cause β-elimination of cysteine in proteins leading to an increase in dehydroalanine and lanthionine (Li et al.

2018). The likelihood of protein degradation and destruction of the intermolecular bonds may also happen at relatively high temperatures, potentially deteriorating protein functionality (Liu and Hsieh 2008). Hence, an optimal temperature needs to be identified which is not too low such that protein denaturation is absent and is not too high so that the denatured proteins undergo further degradation and breakdown.

Temperatures between 95°C and 190°C with 40% to 70% moisture content are known to form fibrous structures. A generalization of temperature ranges corresponding to different moisture levels is not feasible due to variations derived from protein sources, and their functionality as different protein sources can behave differently under similar processing conditions. Readers are encouraged to explore the section on barrel temperatures by Zhang et al. (2019) to get a summary of reported studies that have analyzed the impact of barrel temperature during extrusion on end-product properties. Further, extrusion temperature has a substantial influence on the textural properties (hardness, adhesion, and chewiness), color parameters (L*, a*, b*), and tensile strength of meat analogs. A higher extrusion temperature modifies the secondary structure of proteins (β1+β2 content), reduces the intermolecular hydrogen bond interaction and consequently alters the texture, color, and tensile force of meat analogs (Xiao et al. 2022).

Screw speed: Screw rotation in an extruder not only generates shear force but is also responsible for mixing and conveying the material forward and inducing the alignment of fibers in the flow direction. The screw rotation also leads to viscous dissipation leading to local temperature rise (Emin et al. 2016). The rate of rotation also determines the residence time of the material in the screw, which impacts the extent of anisotropy induced. High shear also leads to lower pressure due to the viscosity reduction of the melt (Shah 2003; Unlu and Faller 2002). The impact of the residence time and the amount of shear experienced by the material is coupled. The optimization of screw speed is critical as screw speeds above or below the optimal range can lead to loss of fibrous structure formation. For high-moisture extrusion, the rotational speed is typically lower (80-100 rpm) than with low-moisture extrusion (300-400 rpm) (Cheftel, Kitagawa, and Queguiner 1992; Zhang, Liu, et al. 2017). As in the case of temperature, the impact of screw speed or shear on material properties is not a simple one and will depend on the protein composition, source, and functionality which determine the rheological properties and phase behavior of the melt. A recent study by Mateen, Mathpati, and Singh (2023) demonstrated the positive effect of barrel temperature and screw speed on the hardness of a whole-cut meat analog product prepared from a blend of soy protein isolate and defatted soya flour. This study showed that the product hardness increases with increasing temperature and screw speed due to the denaturation and agglomeration of protein molecules (Kitabatake et al., 1990). In addition, higher shearing generates excess heat, which can lead to a harder structure. Readers are encouraged to explore the section on screw speed by Zhang et al. (2019) to get a summary of reported studies that have analyzed the impact of screw speed during extrusion on end-product properties.

Decoupling the impact of temperature and screw speed is crucial for determining conditions suitable for obtaining the anisotropy and fibrous texture desired in meat analogs. While the impact of temperature and screw speed can vary depending on the raw material, it helps give mechanistic insights into the phenomena occurring inside an extruder. Closed cavity rheometers are useful instruments for determining the correlations between end-product properties and processing conditions as they can mimic the conditions of extrusions while enabling decoupling temperature and shear to create various samples.

Feed rate: Feed rate impacts the residence time of the material in the barrel and the degree of filling of material in the barrel which dictates the compressive action or compaction and pressure inside the barrel (Unlu and Faller 2002; Maurya and Said 2014). With lower feed rates, the residence time is higher, allowing more protein denaturation, thereby facilitating anisotropic and fibrous structure formation. It is for this reason that high-moisture extrusion has 2-4 times lower feed rate than low moisture extrusion (Thymi et al. 2008; Chen, Wei, and Zhang 2011). Kang (2007) studied the impact of feed rate on various properties of the extrudate and found that lower feed rates were associated with a higher degree of texturization, hardness, chewiness, and color but lower water holding capacity. It should be noted that the impact of feed rate will vary across different extruder designs. An optimal feed rate needs to be determined such that it is not too high, leading to lack of compaction, loss of pressure, and low residence time, and not too low, leading to over-processing and loss of fibrous texture due to long exposure of excessive shear and high temperature.

Scale-up considerations: One of the key challenges every product developer must overcome is scaling up the production process from a lab scale to a pilot scale to a manufacturing scale. With extrusion processing, it is recommended that scale trials should be done from a lab scale (0.5-20 kg/h capacity) to a pilot scale (50-100 kg/h) instead of jumping from a lab scale to a manufacturing scale (500 kg/h). While it may be tempting to maintain process parameters like temperature, screw speeds, specific mechanical energy, and torque, the impact of differences in scale and design differences like clearance, diameter ratio, and L/D ratio, if applicable, will require optimization of process parameters. The heat and mass transfer profile drastically changes as one upgrades from lab scale to manufacturing scale due to a change in material surface area to volume ratio. This impacts the flow as well as the temperature profile inside the barrel and the die. Hence, the temperature profile, pressure profile, residence time that comprise the conditions of bulk material, and the conditions of the material close to the die surface are of relevance for scale-up. Additionally, during lab-scale trials, one needs to ensure there is precise ingredient dosage and process control as even small variations can create a large impact on end-product properties, which may not be the case at the pilot or manufacturing scale.

Impact of formulation

Proteins and moisture: Water in the extrusion process impacts the melt viscosity, facilitates and participates in chemical reactions, impacts temperature and pressure profile, and influences the plasticity and foaming capacity of the material (Chen et al. 2010). Water acts as a plasticizer, reducing the glass transition temperature of the melt and increasing the mobility of protein molecules, thus enabling the physical and chemical transformation of ingredients. Studies have demonstrated that an increase in moisture content from 35% to 50% improves the degree of texturization and adhesiveness while decreasing the chewiness of texturized soy protein (Wei, Zhao, and Kang 2009). Thus, the moisture content impacts the texture profile of the meat analog. Some of the properties impacted by moisture content include hardness, chewiness, water-holding capacity, nitrogen solubility index, and digestibility.

The formation of fibrous structure and development of anisotropy heavily depends on the protein and moisture content in the material in the extruder. Experiments are geared towards exploring new ingredients and new ingredient combinations for creating HMMA involving optimization of moisture and protein content alongside process conditions to arrive at a product with desired technical attributes.

In conjunction with screw speed and feed rate, moisture and protein content influences the specific mechanical energy (SME) of the high-moisture extrusion process. SME increases with screw speed and blend protein concentration and decreases with an increase in moisture and feed rate. The contrasting effects of moisture and protein concentration on SME are likely as both an increase in moisture content and a decrease in protein content would lower the melt viscosity and thereby reduce the SME at the same screw speed. Moisture content and protein concentration are inversely and directly related to melt pressure, respectively (Mateen, Mathpati and Singh 2023). With respect to product parameters, hardness and chewiness increase significantly with protein concentration and moisture content (Lin et al., 2000; Zahari et al., 2021; Zhang et al., 2020).

Carbohydrates: Low molecular weight carbohydrates, like glucose and reducing sugars, participate in the Maillard reaction with free amino acids, which leads to the browning of TVP and modification in taste (Zhang 2007; Wild et al. 2014). The formation of protein-sugar conjugates leads to the formation of insoluble polymeric compounds with distinctive colors (Guerrero et al. 2012). Higher molecular weight carbohydrates, like starch and fibers, can positively impact the formation of fibrous structures. However, it is recommended that the carbohydrate content be less than 10% (Taranto, Kuo, and Rhee 1981; Wang et al. 2002). Starch can undergo degradation to form lower molecular weight carbohydrates which can further react with amino acids as described earlier. Starch is also known to undergo gelatinization at processing temperatures and interact with proteins to prevent unfolding and aggregation and lower the melt viscosity. Some researchers have hypothesized that starch exists as a separate dispersed phase during high moisture extrusion, helping stabilize the hydrophobic interaction of protein molecules while facilitating multi-layer structure formation during the cooling process (Zhang et al. 2016; Smith, Mitchell, and Ledward 1982).

Lipids: Lipids act as plasticizing agents during the extrusion process and should ideally be below 15% in concentration (Schoenlechner and Berghofe 2000; Vaz and Areas 2010; Gwiazda, Noguchi, and Saio 1987). Lipids form complexes with starch and proteins and coat their surfaces, thus reducing friction between the molecules leading to lower shear and protein aggregation (Alzagtat and Alli 2002). Lipids can undergo oxidation and degradation depending on their sensitivity to the process conditions.

pH and ionic strength: Given the high moisture content during the extrusion process, the properties of protein dispersion like hydration, protein-protein interaction, and rheological behavior are susceptible to change as the pH and ionic strength of the system change. The impact of pH, addition of different salts, and ionic strength of the protein dispersion have not been studied extensively, especially for high-moisture extrusion processes. While numerous studies have explored structure formation in dilute protein dispersions (<10% concentration), there is a scope to analyze high concentration systems as in the case of high-moisture extrusion (Loveday et al. 2010; Loveday et al. 2011; Pan and Zhong 2015).

Readers are encouraged to explore the work by Beniwal et al. (2021), which summarizes studies reporting the impact of incorporating ingredients at varying concentrations during high-moisture extrusion.

The creation of meat analogs with anisotropic and fibrous structures can be attributed to the transformation of ingredients at a molecular level and the evolution of rheological properties, phase behavior, and microstructure. An understanding of both transformations is essential to build mechanistic insights into the high-moisture extrusion process and enable prediction and smart selection of ingredients and processing conditions. The process of structure formation in high-moisture extrusion systems has been studied widely by researchers, and various mechanisms have been proposed to explain the same which are discussed in detail in the following sections.

Molecular or chemical transformations during extrusion

During the extrusion process, depending on the protein source, proteins undergo unfolding or denaturing, association, aggregation, and crosslinking (Fig. 6) (Camire 1991; Day and Swanson 2013). The transformation of proteins during the extrusion has been an active area of exploration. Various hypotheses based on findings of different researchers have been discussed in the section below.

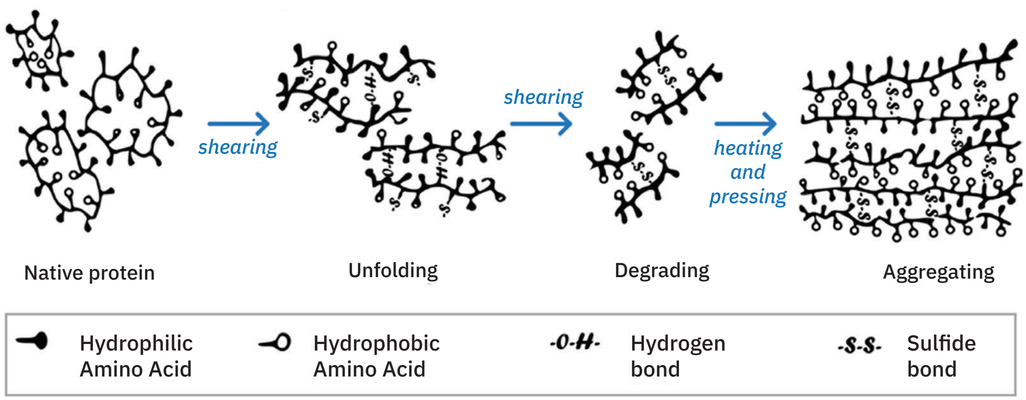

Figure 6. Conformational changes of protein during the extrusion process
(Source: Zahari et al. 2020; Zhang et al. 2018)

As mentioned before, the combination of heat and shear is a prerequisite for the formation of fibrous and anisotropic structures desired in meat analogs. Heat denatures protein and modifies the intermolecular and intramolecular linkages, especially through the weakening of hydrogen bonds (Cordier and Grzesiek 2002) and strengthening of hydrophobic interactions as temperature rises (van Dijk, Hoogeveen, and Abeln 2015). Depending on the type of protein used, there is crosslinking of protein molecules through disulfide bonds. Gluten protein is well known for its ability to form a cross-linked network upon hydration, which is attributed to the formation of disulfide linkages. These disulfide linkages have a significant impact on melt properties during processing as well as end-product texture properties like hardness, chewiness, and springiness. As temperature rises, free thiol groups become reactive (Evans 2001) and existing disulfide linkages become prone to modification (Volkin and Klibanov 1987). Disulfide bonds can undergo beta-elimination catalyzed by alkali to form free thiol groups under neutral pH and high temperature (Volkin and Klibanov 1987). As a result of the presence of free thiol groups and disulfide bonds, a transient reversible network is formed due to thiol-disulfide exchange reactions (Ryle and Sanger 1955; Bloksma 1975; Schofield et al. 1983).

During extrusion, protein-protein interactions undergo changes in conformations especially due to modifications of disulfide bonds, hydrophobic interactions, and hydrogen bonds (Liu and Hsieh 2008; Chen, Wei, and Zhang 2011). Most covalent bonds, especially peptide bonds, remain unaffected by the process (Osen et al. 2015; Shah 2003; Ledward and Tester 1994). The change in protein-protein interactions impacts the viscosity of the melt phase, gelation, water holding capacity, solubility, and other functional properties of the extrudates (Day and Swanson 2013). The degree of modification and type of interactions that are favored during extrusion also depend on the initial conformation of the protein isolate or concentrate. The extraction process, post-extraction treatment, as well as protein source, influence the final chemical and physical properties demonstrated by extruded protein.

Although most studies reported in the literature agree on the impact of disulfide bonds, hydrophobic interactions, and hydrogen bonds on protein aggregation and structuring (Liu and Hsieh 2008; Chen, Wei, and Zhang 2011; Emin and Schuchmann 2017; Pietsch, Karbstein, and Emin 2018), there is a debate on the order of importance of these interactions, which also varies depending on the protein source. Various techniques have been deployed to understand the difference in nature of intramolecular and intermolecular bonding between the feed material and final extrudate. Current limitations of these techniques restrict testing to ambient conditions, thus protein modification during thermo-mechanical processing is restricted. Even with dead stop experiments where the material is sampled from different extrusion zones, the material is examined after cooling. Thus, such studies cannot be used to estimate the changes that occur during the processing as cooling may lead to changes in material properties. Decoupling the impact of actual processing conditions from the effect of cooling the material is a challenge. Inline measurement of the sample at processing conditions through appropriate equipment or offline measurement using analytical tools that mimic process conditions can be a potential solution and will be important to correlate the functionality of protein to process condition.

Measurement of interaction effects: The most commonly used method involves using various buffer and reagent systems to solubilize protein (Samard, Gu, and Ryu 2019; Chiang et al. 2019; Zhang et al. 2020; Pietsch et al. 2019; Osen et al. 2015; Fang, Zhang, and Wei 2014; Liu and Hsieh 2007). The buffer and reagent systems are chosen such that they selectively disrupt different types of protein-protein interactions making them soluble. Phosphate buffers are used to extract soluble protein without disrupting any interactions. Urea is used to disrupt physical interactions like hydrophobic, ionic, and hydrogen bonds, while dithiothreitol (DTT) is used to reduce disulfide bonds to free thiol groups (Liu and Hsieh 2007). Using buffer systems and particular choice of reagents has been a topic of controversy amongst researchers. A new combination and order of addition of reagents were explored by Liu and Hsieh (2008), where instead of adding different reagents to the baseline buffer, the researchers proposed the removal of reducing agents from the buffer system. To measure protein-protein interactions based on the differences in molecular weights between the feed material and end-product, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Chen, Wei, and Zhang 2011; Fang, Zhang, and Wei 2014; Osen et al. 2015; Zhang et al. 2020) or size exclusion chromatography (SEC) (Strecker et al. 1995; Domenek et al. 2002; Fang et al. 2013; Pietsch, Karbstein, and Emin 2018) can be conducted.

Spectroscopic techniques can potentially monitor the molecular characteristics of materials at various stages of extrusion. Raman spectroscopy, for example, can provide insights into disulfide bond formation and differentiate between exposed and buried thiol groups (Wang et al. 2016; Ozakisj, Mizunolt, and Iriyamas 1987). Spectroscopic tools like Raman and Fourier Transform Near-Infrared Spectroscopy (FT-NIR) have already been used to study the melt properties during the processing of non-food polymers (Barnes et al. 2007). Extension of the application of these tools for extrusion can pave the way to generate insights into the nature of physical and chemical bonds and their modification during extrusion processing.

Rheological, phase, and microstructural transformations during extrusion

Most hypotheses explaining the change in physical and rheological properties that facilitate structure formation consider the structuring process to occur in the cooling die due to the relative motion of multi-phase systems (Akdogan 1999; Tolstoguzov 1993; Cheftel, Kitagawa, and Queguiner 1992; Murillo et al. 2019). However, there are varied theories on the type of multi-phase system, its composition, the formation of the multi-phase system, and the driving force for relative motion (Cornet et al. 2022).

Laminar flow and flow-induced structuring: Flow-induced structuring has been identified as a critical mechanism for structure formation (Zhang et al. 2019; Akdogan 1999; Osen et al. 2014; Palanisamy et al. 2019; Murillo et al. 2019). The typical extrusion process for proteins can be assumed to have a low Reynolds number for the given flow rate, density, and viscosity of the melt. Cornet et al. (2022) evaluated various experimental data reported in the literature for high moisture extrusion and proposed the Reynolds number to be in the range of 2 to 73 for a melt viscosity of 1kPa. At this Reynolds number, flow can be assumed to be laminar (Metzner and Reed 1955). This laminar flow is where the flow rate of melt varies between the center of the die to the surface of the die in such a way that the material at the center moves the fastest while the material closest to the surface moves the slowest. The exact velocity profile within the laminar flow remains to be studied and explored. The shear flow due to an increase in viscosity as the material cools down has been attributed to the induction of fiber alignment in the die section. The differences in viscosities of material at the center of the die and near the surface are likely to further amplify the velocity gradient leading to fiber formation (Cheftel, Kitagawa, and Queguiner 1992; Akdogan 1999). While this mechanism explains fiber formation from a fluid mechanics perspective, direct evidence to validate this hypothesis remains to be explained and further rheological investigations can help provide data to verify the validity of this hypothesis.

Phase deformation and alignment: Another hypothesis that was extensively explored in the 1990s include several studies by Tolstoguzov's group. The researchers drew an analogy between a multi-phase emulsion system and material formed in the extrusion process (Tolstoguzov 1993; Grinberg and Tolstoguzov 1997; Polyakov, Grinberg, and Tolstoguzov 1997). As with emulsions, the dispersed phase undergoes deformation under shear flow and aligns in the direction of shear flow, wherein the extent of deformation is a function of the ratio of the viscosity of dispersed phase and continuous phase (Tolstoguzov, Mzhel'sky, and Gulov 1974). This mechanism was extrapolated to explain structuring in a cooling die for extrusion (Tolstoguzov 1993). The phase deformation and alignment due to shear followed by cooling were hypothesized to freeze the structure in a way that resembled fibrous structures. The coalescence of deformed dispersed droplets was assumed to lead to the formation of long and short fibers. More recently Dekkers et al. (2016) used this mechanism to explain structure formation using soy protein isolate and pectin in a shear cell. Validation of these mechanisms rests on creating/using analytic techniques that can not only identify phases in these systems but eventually measure the changes in phase behavior.

Two-phase interactions: The phase behavior of melts formed during extrusion have been studied extensively using experimental and theoretical tools (Grinberg and Tolstoguzov 1997; Polyakov, Grinberg, and Tolstoguzov 1997; Tolstoguzov 1993; Cheftel, Kitagawa, and Queguiner 1992; Grabowska et al. 2014; Dekkers, de Kort, et al. 2016; Schreuders et al. 2020; Cornet et al. 2022). Protein-polysaccharides, protein-protein, protein-water, and other ingredient mixtures have been hypothesized to cause phase separation. The presence of separate phases in protein-protein mixtures was verified experimentally using NMR and rheological methods for hydrated mixtures of pea protein, soy protein, and gluten (Dekkers, de Kort, et al. 2016; Schreuders et al. 2020). The theory that similarities in rheological properties of different phases at high temperatures may be responsible for fiber formation has also been explored (Dekkers, Emin, et al. 2018). However, Schreuders et al. (2020) have demonstrated that it is possible to create fibrous structures using a blend of pea protein and gluten despite differences in their rheological properties. Formation of fibrous structures from single ingredients like gluten (in the shear cell) (Grabowska et al. 2014), soy protein isolate (Fang, Zhang, and Wei 2014), and pea protein isolate (in high moisture extruders) (Osen et al. 2015) have also been demonstrated in the literature. Although protein isolates are assumed to behave as single-phase, the above studies indicate that multi-protein ingredients within a protein isolate may lead to the formation of two-phase systems with insoluble components forming a separate phase as proposed (Arêas 1992). In addition to proteins, polysaccharides can also contribute to the formation of a separate phase.

Spinodal decomposition: Some researchers consider the melt as a homogeneous phase, to begin with, which subsequently undergoes phase separation upon cooling (Ledward and Tester 1994; Murillo et al. 2019). The hypothesis proposed is that the melt of protein isolate and water phase separate from water-rich and protein-rich domains due to spinodal decomposition upon cooling, driven by a temperature drop. The assumption that melt is a single-phase homogeneous material before the cooling process contradicts the thermodynamic incompatibility of proteins since protein isolates are mixtures of different proteins. However, the numerical modeling by Murillo et al. (2019) is the first of its kind in providing insights into fiber formation during extrusion. More research that combines experimental studies with numerical modeling will provide mechanistic insights and eventually pave the way to predictive modeling.

Other theories: Given the complexity of food systems, it may be beneficial to study simpler systems to gain an understanding of mechanisms of action. Similar to HME, shear-induced structure formation is observed in particle suspensions and two-phase polymer systems. In the case of suspension, shear bands are observed and attributed to structure formation (Scirocco, Vermant, and Mewis 2004; Pasquino et al. 2010; Van Loon et al. 2014; Santos De Oliveira et al. 2011; Divouxet al. 2016; Vermant and Solomon 2005). Van Loon et al. (2014) identified that the shear thinning behavior of the continuous phase was essential for the formation and stability of shear bands. Although shear-thinning fluid systems are not representative of suspension or two-phase polymer systems, polymer melts are typically shear-thinning in nature. Thus, in the continuous and dispersed phases, both will likely have similar properties. Approximating the dispersed phase as rigid hard particles will be an oversimplification, but it can give some mechanistic insights into structuring. Similarly, these model systems have been evaluated at low polymer concentrations (10%) compared to 30-40% for HMMA. Although shear-band formation has been reported for sheared polymer solutions (Caserta and Guido 2012; Cromer et al. 2013; Caserta, Simeone, and Guido 2008; Migler 2001), the presence of shear-bands and their impact, and the extent of influence on the structuring process remain unclear. The use of simplified model systems can give insights from a rheological perspective and should be explored for advancing the understanding of high moisture extrusion processes.

Measurement of rheological properties: The rheological characteristics of the material in various extrusion zones can be captured through various techniques. Inline rheological measurement utilizes capillary or slit die rheometers to measure the viscosity of the material in steady-state conditions (Thadavathi, Wassén, and Kádár 2019). The slit die rheometer is attached to the end of the extruder in place of the extruder die, and the pressure drop is measured and used to calculate shear stress at the wall. The wall shear rate is subsequently calculated using material velocity. Using a series of dies with a range of diameters, the flow curve for the material can be determined. The use of dies of varying diameters can lead to modification in back pressure and flow characteristics (Emin et al. 2017). Designing an adjustable geometry to maintain the back pressure can ensure material characteristics are not impacted (Horvat et al. 2013). Studies that have reported the use of capillary rheometers for determining the properties of food materials and polymer melts include Corfield et al. (1999), Aichholzer and Fritz (1998), Ralston and Osswald (2008), Ponrajan et al. (2020), and Beck et al. (2017).

While off-line rheological methods do not offer insights into processing characteristics, they enable large sample analysis and a greater understanding of the impact of ingredients. Tools such as oscillatory shear rheometry, capillary rheometry, and viscometry have been used for off-line rheological measurements; these can be used to compare the properties of various ingredients/feed materials through measurement of parameters such as viscosity, elastic modulus, viscous modulus, and elongation viscosity. Since conventional bench-top rheometers cannot provide high pressure, temperature, and shear rates, these do not mimic extrusion processes and hence cannot be used to accurately draw comparisons between end products and different ingredients. Rheometers such as capillary flow rheometers that have been used to measure at high temperatures and pressure have also been used for the measurement of melt characteristics in plastics and bioplastics.

Closed cavity rheometers (CCR), traditionally used to analyze the vulcanization of rubber, have recently been used for the measurement of food polymer rheology (Pommet et al. 2004; Emin et al. 2017; Pietsch et al. 2019; Geerts et al. 2018; Dekkers, Emin, et al. 2018; Schreuders et al. 2020). These rheometers can withstand high temperatures and pressures without moisture loss, making the measurement process close to extrusion processing conditions. Since these rheometers can only provide oscillatory deformation, instead of rotation, there are limitations in mimicking the flow patterns within the extruder barrel or shear cell. Studies reporting the use of CCRs for the measurement of rheological properties of food polymers produced through extrusion or shear cell technology include Pommet et al. (2004), Geerts et al. (2018), Dekkers, Boom, et al. (2018), Schreuders et al. (2020), Pietsch, Karbstein, and Emin (2018), and Pietsch et al. (2019). CCRs can be used for the sample preparation of various ingredients for preliminary analysis. CCRs also help decouple the impact of temperature and shear to understand their impact on changes in material properties.

Another rheological method that has been recently reported for understanding melt properties beyond the linear viscoelastic regime involves the usage of large amplitude oscillatory shear rheology (LAOS). Schreuders et al. (2021) used this technique to find that thermal treatment caused a loss of elastic properties in pea protein isolate faster than in the case of soy protein isolate and gluten. The use of a plethora of rheological techniques and the recent utilization of CCR and LAOS need to be further investigated to interpret the results and map the measured parameters to the structuring process to build deeper mechanistic insights into thermo-mechanical processing systems.

Plant-based meat: Technical evaluation and sensory analysis

Consumer acceptance of plant-based meat products vastly depends on how closely the sensorial experience of eating the product resembles that of animal meat. Hence, this is the key driving factor for optimizing product design. Various attributes can be evaluated for qualitative and quantitative descriptions of this sensory experience. However, sensory studies can be expensive, time-consuming, and have longer feedback loops for product development. For new product development, it is essential to benchmark prototypes against reference products on quantifiable properties. These quantifiable properties should ideally indicate sensory attributes evaluated during consumer studies to provide feedback to product development. Typically, in any food product development process, the preliminary screening of prototypes is based on technical assessment, which involves using analytical and instrumental techniques. The next level of screening can be done using an untrained consumer panel or a trained consumer panel for sensory assessment, if available. The final level of selection is carried out by a large-scale representative consumer sample. At every level of screening, consumer feedback can provide directional inputs on improving product profiles.

For existing food products, the technical parameters as well as sensory parameters are well known within the industry and are standardized. However, these standard test methods need to be defined for plant-based meat. Some of these standards can be adapted from the animal meat industry. It is also important to note that the technical parameters of plant-based meat should indicate sensory attributes relevant to consumers.

Technical evaluation of plant-based meat

The typical technical properties of interest indicate texture, fibrous structure, anisotropy, color, visual appearance, and to some extent, mouthfeel. Flavor profiles are not typically evaluated during the technical evaluation stage but by expert sensory panels as well as untrained consumer panels. Analytical techniques and measurement protocols are deployed to measure composition, protein digestibility, essential amino acid profile, nitrogen solubility index, texture profile, microstructure, dynamic rheological properties, water holding capacity, and color. Of these measurements, the first four correspond to the determination of nutritional value, and the rest can be related to consumer-relevant sensory attributes. Other techniques are deployed to understand the mechanism of action or the process of fiber and anisotropic structure formation. These include protein characterization through spectroscopic techniques, microstructure analysis through imaging techniques, rheological measurements, and protein-protein interactions by measuring solubility in various buffer systems and analysis of molecular weights before and after thermo-mechanical processing using sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Some of these techniques will be discussed in detail in the section on the mechanism of action.

Texture analysis: Texture is defined as 'a combination of the rheological and structure (geometrical and surface) attributes of a food product perceptible by means of mechanical, tactile, and where appropriate, visual and auditory receptors' (ISO, 2008). Texture profile analysis is one of the most important techniques used to evaluate the quality of meat analogs. Its application is not just limited to academia but is critical for product developers. Texture profile analysis involves measuring the material properties in response to the application of normal force or force perpendicular to the surface of the product of interest. The equipment, known as a texture profile analyzer, measures the force required to compress or elongate a material. The material properties measured by the texture analyzer include hardness, tensile strength, adhesiveness, brittleness, springiness, and gumminess. These parameters can be compared head-on with animal meat products, and feedback can be used to optimize process conditions or ingredient blends. Also, elongation tests indicate the extent of anisotropy in the extrudates. A typical texture profile analysis or double compression test involves compression of a sample of standard geometry followed by relaxation twice between parallel plates with a diameter larger than the sample at a defined speed. This process is meant to replicate a two-bite chewing process making it relevant for the analysis of meat analogs (Nishinari, Fang, and Rosenthal 2019). Parameters measured by texture analyzers include hardness (maximum force during the first compression peak), resilience (downstroke area under the first compression peak/upstroke area under the second compression peak), springiness (time to reach the peak during the second compression/time to reach the peak during the first compression), and chewiness (hardness × springiness × area under the second compression peak/ area under the first compression peak) (Mattice and Marangoni 2020). In simple terms, hardness is a measurement of how firm the product is, springiness shows how well the product returns to its original structure after the first compression, resilience describes how well the product regains its original height after the compression, and chewiness shows how much work is needed to chew the product to achieve the texture that is suitable for ingestion. The diagram below (Fig. 7) represents the measured attributes obtained from a texture profile analyzer.

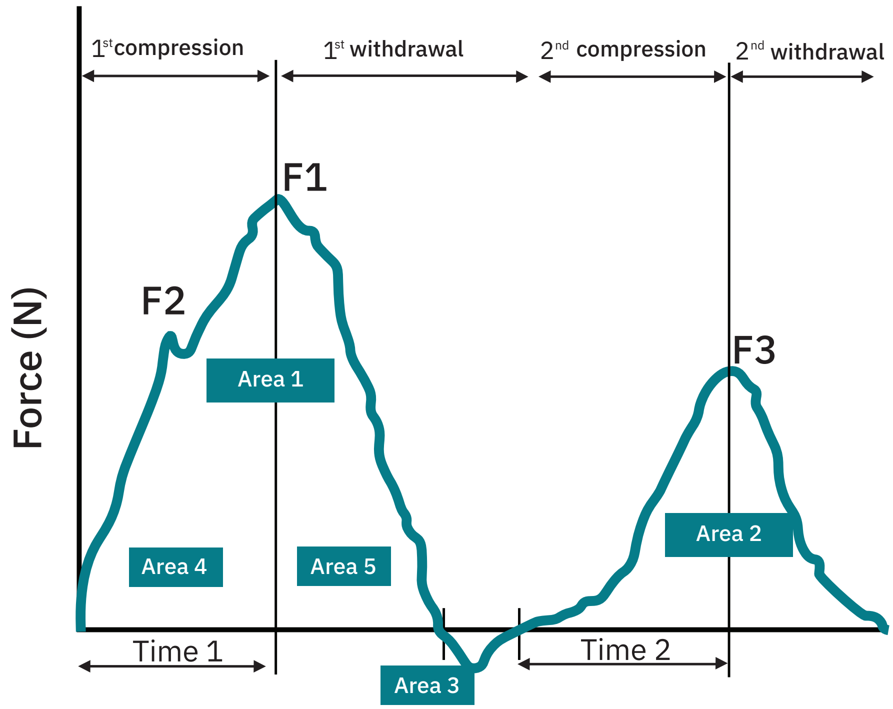

Figure 7. Graph obtained after texture profile analysis

Here: Hardness (N) = F1, Adhesiveness (N.s) = Area 3, Fracturability (N) = F2, Cohesiveness = Area 2/Area 1, Springiness (%) = Time2/Time1*100, Chewiness (N) = F1*Time 2/Time 1*Area 2/Area 1

Apart from texture profile analysis tests, tensile tests, and cutting strength, measurements can also be performed using a texture analyzer and are commonly reported in the literature; note that various studies may use different measurement geometries, test protocols, and fixtures for the analysis. Cutting force and tensile tests are used to determine the extent of fiber formation and strength of the structure and can be linked to determining the quality of meat in terms of attributes like tenderness. Samples can be cut in a standard configuration and subjected to cutting force through a knife-shaped geometry that moves down at constant speed. The force experienced by the knife at different depths (for both longitudinal and transversal cuts to the outflow direction from the extruder) is noted, and the maximum cutting force or force at a fixed depth is reported.

While using a texture profile analyzer, it must be noted that both the compression and the cutting tools can be used to report some common parameters like hardness. However, compression tools should be preferred for measuring these parameters as it is expected to provide more reliable data due to the larger size of the tool used compared to the sample. Also, while comparing parameters across studies, the tool used for parameter measurement should be considered.

Tensile stress tests are performed to measure the degree of anisotropy in meat analogs indicated by the anisotropy index. The sample is cut in pieces, exposing the parallel and perpendicular surface to the shear flow, and these are subjected to a constant deformation rate. The anisotropy index is the difference between the maximum stress value resulting from parallel deformation and the maximum stress resulting from perpendicular deformation (Krintiras et al. 2014; Krintiras et al. 2016). It is, however, not sufficient to measure tensile strength to determine anisotropy, as some case samples with a high anisotropy index have been found to have a low degree of fiber structure formation (Krintiras et al. 2015). Hence, visual observation and microscopy techniques need to be used for a complete understanding of fibrous structure formation.

Visual and microstructural characterization: Visual analysis of meat analogs includes comparing photographic images and using techniques like color measurement, microscopic imaging, and image processing which provide objective criteria for evaluating and comparing samples. While photographs can provide some insight into fibrous structure formation, image processing of high-resolution photographs of analog meat cuts exposing the parallel and perpendicular surface can enable the detection of fibrous structures and the quantification of the length, width, and density of fibers formed. The color of meat analogs has not been a focus area due to relatively higher challenges in creating textured meat analogs and the relevance of fibrous structure from a consumer acceptability perspective. The color of extrudates has been measured to evaluate the impact of the cooking process and structuring on color and are typically measured using a colorimeter that uses CIELAB color space. Maillard reactions and degradation of pigments in the extruder are responsible for changes in color during the extrusion process.

Microstructure analysis techniques include scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM), transmission electron microscopes (TEM), atomic force microscopy (AFM), and optical microscopy. These techniques can be used to construct images at different length scales, and these images can be subsequently processed for quantitative assessment of color, shape, porosity, and surface texture. Such data can provide insights into structure formation and changes in physical characteristics of raw material after processing (Krintiras et al. 2015; Azzollini et al. 2019; Chiang et al. 2019; Schreuders et al. 2019; Samard and Ryu 2019; Palanisamy et al. 2019; Caporgno et al. 2020; Yuliarti, Kovis, and Yi 2021).

Academic researchers have widely used SEM to understand the microscopic structure. CLSM has the potential to create 3D images of samples with the ability to identify components like fats and proteins and their structure through the use of fluorescence dyes (Schreuders et al. 2019; Dreher et al. 2020).

In addition to the above analytical techniques, other commonly used characterization techniques include measurement of water holding capacity, integrity index, moisture, and cooking loss.

Water holding capacity and cooking loss (physical property): The ability of meat to bind water and release water during mastication contributes to the juiciness perceived during meat consumption. Hence, it is critical to measure the water-binding capacity of meat analogs. Water holding capacity is calculated as the percentage of water lost per gram of the sample after it is subjected to centrifugation. Similarly, cooking loss is measured as the percentage of water released per gram of initial sample weight during a standard cooking process.

Molecular characterization: Spectroscopic techniques such as infrared, Raman, fluorescence polarization, NMR, circular dichroism, and light scattering provide insight into the local composition at the surface of the product, intermolecular interactions, nature of bonds, and anisotropy in meat analogs.

Unlike other techniques, spectroscopy is non-invasive and requires only small samples for analysis. Other techniques may also be deployed to characterize the end product, like particle size distribution and zeta potential, which may be more relevant to non-solid systems like dairy alternatives. Hence, these have not been discussed in this report.

Digital processing of oral response: A novel oral processing technique, namely, electromyography has been proposed to understand chewing behavior and bridge the gap between texture profile analysis and sensory trials. The ability to replicate consumer behavior provides a consumer-relevant standard and reproducible process that can be used to evaluate differences in various prototypes as well as between plant-based meat analogs and animal meat. Jaw tracking and muscle activity tracking using electromyography are the main components of the measurement system. These systems are used to calculate parameters like the number of chews, chewing frequency, and chewing cycle duration (Çakır et al. 2012). This data will be helpful to get deeper insights into the mastication process and correlate material properties like hardness and fibrous structures to sensorial characteristics like perceived firmness and chewiness.

Rheological analysis: Rheological techniques are most relevant for understanding the impact of processing conditions on creating anisotropic and fibrous structures. These can also be used to compare the rheological properties of the benchmark, animal-based meat, and meat analog. Rotational rheometers like controlled strain and controlled stress instruments, capillary rheometers, and closed cavity rheometers have been used for this purpose. The stiffness and elasticity of the material can be determined by measuring Young's modulus using rheometers.

Closed cavity rheometers can closely replicate the conditions of an extruder as it is possible to achieve high temperature and pressure without moisture loss while maintaining even shear throughout the sample due to the bi-conical design (Emin and Schuchmann 2017; Dekkers, Boom, and van der Goot 2018). Rheometers can provide mechanistic insights into the structural properties of meat analogs, the formation of fibrous structures, and induction of anisotropy, and the role of temperature and shear information.

Some of the spectroscopic and imaging techniques described may not be readily available to use at the lab scale or pilot scale for those looking for commercial production of either extrudates or end products. Such analyses are performed by types of equipment that are likely to be available in research laboratories or academic institutions, where the focus is to develop a basic understanding of the changes in physical and chemical characteristics of the material. Depending on the availability of equipment, costs associated with procuring them, and relevance at a manufacturing scale, the use of some of these techniques might not be required for product development and commercialization. Techniques like texture profile analyzer, colorimetry, tensile tests, and visual analysis techniques are more relevant to compare lab scale and pilot scale prototypes. Readers are encouraged to explore the work by Schreuders et al. (2020), which provides an in-depth review of technologies available for the characterization of meat and meat analogs.

Sensory analysis

Sensory analysis is the closest test to predict consumer acceptance of meat analogs. Data on consumer behavior indicates that taste and convenience are the key drivers for adopting plant-based meat products. Particularly for India, GFI India's consumer study of the early adopter cohort indicated that early adopters are positively disposed towards the alternative meat, egg, dairy, and seafood categories and rate alternate meat higher on health, taste, access, and ethics over conventional meat. While this is all encouraging data, the real success of plant-based meat, egg, dairy, and seafood depends on the quality of products available for consumers. Sensory evaluations, hence, play a key role in providing feedback on whether a product will succeed in the market. These evaluations also help identify product attributes and properties that contribute to product acceptability and elements of consumer delight. At some point, mapping these consumer-relevant attributes to properties measured by instruments will help stimulate or predict consumer acceptability for various recipes and process conditions.

Sensory attributes include flavor, taste, mouthfeel, and texture profile during consumption, and also pre-consumption visual and olfactory cues like shape, color, appearance, and smell. The relative importance of various attributes pre-consumption and during consumption depends on the product format (ready to cook, ready to eat, uncooked), product type (first generation, second generation, third generation, type of meat), and consumer preferences depending on cultural and regional nuances. Sensory studies can be classified into three categories - discrimination or difference testing, descriptive testing, and affective testing. Most studies deployed for evaluating meat analogs are either descriptive or affective tests (Lawless and Heymann 2010).

Descriptive tests: In a descriptive sensory analysis, panelists are asked to provide a detailed qualitative and quantitative assessment of a product's sensory profile. Descriptors like 'fibrousness', 'firmness/hardness', 'juiciness', 'elasticity', 'beany', 'brittleness', 'earthy', 'chicken', 'crumbly', 'moist', 'tenderness', and more general attributes, such as 'taste', 'flavor', and 'smell' are provided to consumers who rate the intensity of each attribute in the product on a standard scale (Lin, Huff, and Hsieh 2002; Savadkoohi et al. 2014; Grahl et al. 2018; Stephan et al. 2018; Chiang et al. 2019; Palanisamy et al. 2019; Taylor et al. 2020). It is important to note that panelists must be trained before attributing ratings to various descriptors. Typically, 8-12 panelists undergo rigorous training to ensure standardization and benchmarking of ratings across panelists (Lawless and Heymann 2010). The descriptive analysis relies on preliminary consumer data to obtain the set of consumer-relevant attributes or descriptors that are quantified for a given product by panelists.

Affective tests: Affective tests, also known as hedonic or consumer acceptability tests, evaluate the degree of liking based on the product's sensory profile. Typically untrained participants in large numbers (>100) representative of the target consumers perform sensory evaluations (Lawless and Heymann 2010). Participants are asked to indicate the extent of like or dislike for the product on a set of sensory properties that capture appearance, flavor, taste, texture, and overall product acceptance. A nine-point hedonic scale ranging from 'like extremely' to 'dislike extremely' is typically used (Lawless and Heymann 2010). Other scales like the 'visual analog scale', 'check-all-that-apply' (CATA), and 'just about right' can be used depending on the comparison required between different prototypes as well as between benchmarks and prototypes. Affective tests have been used to assess the acceptance of meat analogs produced from peas, wheat, peanut, chickpea, mycoprotein, and soy protein (Rehrah et al. 2009; Kim et al. 2011; Savadkoohi et al. 2014; Yuliarti, Kovis, and Yi 2021; Gómez, Ibañez, and Beriain 2019).

Fiorentini, Kinchla, and Nolden (2020) present a comprehensive review of sensory studies reported in the literature. Most sensory studies conducted thus far have focused on evaluating the impact of including minor ingredients like hydrocolloids, flavoring agents, and salt on the sensory profile of meat analogs. Few studies have explored the impact of various ingredient blends on the sensory properties of TVP or HMMA. With new ingredients and blends being explored, it is critical to evaluate the sensory profile of extrudates, especially high-moisture meat analogs aimed at mimicking whole-cut meat.

Several opportunities could be explored in sensory testing to accelerate the development of plant-based meat analogs:

1. Standard sample preparation technique: Within a particular study, the sample preparation technique is expected to be the same for all samples. However, to compare data reported across different studies, a standard protocol of sample preparation for each type of instrumental test for a particular analytical test, and for sensory evaluations should be adopted.

2. Choice of benchmark: Most sensory studies and technical characterization studies do not use an animal meat product in the set of evaluated products. The presence of a corresponding animal meat product can serve as a benchmark to determine key differences between the characteristics and sensory profile of the prototype and the actual meat product that is aimed to be mimicked. The feedback from such data provides quantitative and qualitative directions to identify the target parameters that need to be optimized during product development.

3. Product format: Depending on whether a product is ready to eat or cook, the sensory evaluation will be impacted by the presence or absence of flavoring agents for the same prototype used. Hence, for sensory evaluation of HMMAs, prototypes can be prepared so that both pre-flavoring and post-flavoring profiles can be captured. If the objective is to identify the role of ingredients and process conditions in the high-moisture extrusion process on product properties, evaluating unflavored prototypes rather than flavored ones will provide a better understanding.

4. Preferences study: Most studies have evaluated consumer acceptability which is different from preference. Since the success of plant-based meat and the alternative protein sector rests on the ability to match or mimic the taste, texture, flavor, and hedonic experience of eating animal meat, it is crucial to evaluate plant-based meat prototypes against animal meat. Thus, discrimination or difference testing should be deployed to evaluate the performance of various plant-based meat analogies.

5. Calibration of descriptors: The set of attributes used for describing properties like plant-based meat varies across various sensorial studies. Creating a set of standard sensory properties and their quantitative descriptions for meat analogs will help compare studies across different

research reports. Some of these can also be adapted or adopted from the meat industry. It is important to ensure that these properties are consumer-relevant and should be determined and validated through a consumer study. It will be interesting to understand how these descriptors change depending on whether the target user is a consumer, customer (TVP supplier), or chef.

For the recommendations of creating various standards, it should be noted that the choice of meat (chicken, pork, beef, mutton), the type of end-product (generation), and the format (ready-to-eat, ready-to-cook) will also impact the set of protocols used for sample preparation, standard descriptors to evaluate sensory attributes and the determination of the benchmark.

Considerations for setting up a manufacturing facility

The choice of setting up a high-moisture extrusion facility can be a perplexing one: from selecting the right equipment design to estimating the project costs to knowing what it takes to run the manufacturing facility. The process is a big black box. GFI India interviewed various stakeholders working in this domain and has come up with broad guidelines that one needs to consider to ease the decision-making process for entrepreneurs and businesses looking to invest in high-moisture extrusion facilities in India.

A few of the questions that need to be addressed include:

- What type of extruder is required?
- From where should the extruder be sourced?
- What kind of ancillary equipment would be needed?
- What would be the typical project cost of setting up a manufacturing facility?

Choice of extruders

The type of extruder used depends on the target end application. If the aim is to create first and second-generation products, one can explore both single-screw and twin-screw extruders for low-moisture extrusion to create dry texturized vegetable protein. In the case of single-screw extruders, a wide range of operating conditions may not be available, and the raw material used for creating dry TVPs can be challenging to handle. The relative mechanical energy input that can be provided in a twin-screw extruder is higher than in a single-screw extruder system. One can also utilize high-moisture extrusion by twin-screw extruders to develop first and second-generation products, but this may not be cost-effective.

For creating third-generation or whole-cut meat analogs, high-moisture extrusion using twin-screw extruders is recommended. However, hydrated dry TVPs obtained from low-moisture extrusion using twin-screw extruders can also be explored. The trade-off between taste and texture profile as a result of the choice of technology will depend on the specific requirements and constraints of the manufacturer.

Once the type of equipment to be used is determined, the next step is to determine the supplier and the required equipment design. In general, for TVP production, a twin-screw extruder is recommended. As explained in previous sections, the key difference between high-moisture and low-moisture extrusion is the presence of a cooling die in the former. Major extruder manufacturers like Wenger, Coperion, Buhler, and Clextral have deep technical expertise in equipment design and optimizing processing conditions, ingredient usage, and other process and maintenance-related aspects.

Cost of extruders

The typical capacity of manufacturing scale equipment is 500 kg/h. In some exceptional cases, 1000 kg/h capacity equipment can also be bought. The cost for such a piece of equipment would be between 0.9-1.6 million Euros. Installation, freight, insurance, and import duties would additionally be applicable and can increase the final cost by 10-30%. Also, equipment costs may differ if negotiated depending on the supplier and exact requirements.

When considering buying a manufacturing scale twin-screw extruder, one needs to consider the following factors:

1. Extrusion expertise: Do you have in-house expertise in using twin-screw extruders?
   If the expertise is limited, it is best to rely on manufacturers who can be knowledge partners and provide long-term technical support. Suppose a strong technical expert with significant experience working on twin-screw extruders is available who can determine equipment design and specifications based on their knowledge. In that case, the custom design of extruders through manufacturers based in China can be a possibility. However, a precise understanding of equipment, processing, and ingredients must be ensured to explore this route. While some Chinese manufacturers can provide equipment at about one-tenth of the equipment cost from established manufacturers, additional costs from installation, maintenance, service support, and quality must be accounted for. This is not to say that equipment sourced from China would lack quality; good quality equipment would still cost no less than 30% of standard equipment suppliers. However, the major drawback will be the lack of service and processing support in the long term.

2. Existing infrastructure: Do you have an existing twin-screw extruder system for producing dry TVPs?
   If yes, then, one can explore customization to include a cooling die setup with existing manufacturers. Suppose a twin-screw extruder is available and is currently used for other food applications. In that case, one can either onboard an extrusion expert or technology consultant, or partner with the supplier to optimize processing conditions for producing dry TVP. If customization is possible then the addition of a cooling die for producing wet TVP can be considered.

3. Budget: How much money are you ready to invest in setting up a manufacturing facility?

For 8 tons/day production volume, an investment in the range of 15 to 40 crores as project costs should be accounted for at the minimum. Additional costs associated with operations, raw material procurement, and distribution would further need to be considered.

Beyond extruders

In addition to the extruder, the manufacturing facility would require installing a feeding system and hot water and cooling water system to support the extrusion operation. Quality control and rejection systems, hot cutting systems, and freezing systems are additionally required for monitoring quality, shaping, and storing extrudates coming out of the die. Kitchen processing equipment like Hobart mixers, grinders or choppers, weighing balances, basic molds, convection ovens, and induction burners and pans are essential for downstream product development purposes. These systems would contribute to an additional 5 to 10% of extruder cost. One would need to account for the costs demanded by analytical equipment for quality measurements like texture profile analyzer, rheometer, and incubators with variable temperatures and humidity as well as water storage, treatment, and purification systems. Typically the project cost would be around 3-5 times the cost of an extruder or even higher in some cases.

Insights from a closed-door roundtable discussion

To install a manufacturing capacity for high-moisture extrusion in India, one needs to address the problem from a systems lens. Hence, to get a holistic perspective on inter-dependent challenges, GFI India organized a roundtable discussion with key stakeholders working on ingredient development, high moisture extrusion facilities, product development, and equipment manufacturing to explore key challenges and potential solutions. The roundtable discussion brought together experts from diverse backgrounds ranging from academic and industry experts with experience working with high moisture extrusion technology to industry players and entrepreneurs working actively to create next-generation ingredients, TVPs, and end products for the Indian alternative protein sector.

Panelists touched upon several challenges that need to be addressed and proposed various opportunities across academic research, ingredient development, supply chain, building manufacturing capacity, and product development. Stakeholders emphasized that academic research is a must for innovation in creating HMMAs. Food products such as chocolate and ice cream that require structuring have evolved over the years because of the scientific knowledge built on aspects like phase behavior, rheology, and structuring processes. More specifically, to meet consumer expectations on attributes like texture, sensory, and nutrition, as well as specific taste profiles in the Indian market, one needs to decipher consumer lingo and link it closely to instrumental measurements performed during product development.

On the ingredient development front, stakeholders across the board agreed that India has a unique advantage in terms of the abundance of crop types and availability of raw materials, which can cater to diverse functional properties and taste and nutritional profiles desirable in an end-product. However, the ability to source raw material of consistent quality at adequate volumes with specifications that provide data on functionality, nutritional value, composition, and, if possible, the flavor profile will be critical to enable large-scale production of TVP. The development of such ingredients is underway as indicated by some of the stakeholders who are focused on designing ingredients from a holistic perspective taking into account flavor, texture, color, taste, nutritional profiles, and functionality. These stakeholders highlighted that the ingredient development process and extrusion processing should also be robust to manage the variation in raw material properties manifested as a result of seasonal changes and differences in agro-climatic conditions.

On the high-moisture extrusion front, stakeholders highlighted that understanding the underlying mechanism of texturization from a processing and ingredient transformation perspective is critical for product development. Established manufacturers of twin-screw extruders have developed this expertise and can provide support in process optimization to customers who purchase their equipment. One of the stakeholders indicated their interest in India and China as emerging markets and is actively exploring setting up an application center in the near future. While relying on the expertise and support of extruder manufacturers is one route to go about product development, one of the stakeholders shared their experience of designing their twin-screw extruder as per their requirements. The key enabler for this particular stakeholder was their deep expertise and experience in extrusion technology, appetite to experiment, going back to the fundamentals of extrusion, and utilizing knowledge from allied fields like plastic extrusion systems to not only design their equipment but also optimize their process for a variety of end applications. Along similar lines, another stakeholder shared an example of a company utilizing a low-cost extruder system and customizing it according to their requirements. However, the stakeholder highlighted the process, material, and food safety risks that must be accounted for while building extrusion systems from scratch.

From a product development perspective, stakeholders echoed the need for multi-stakeholder partnerships to create a mature supply chain from crop procurement to product development. Stakeholders emphasized the need to create financing and investment vehicles that can provide access to capital for entrepreneurs. Financing options available through traditional financial institutions, as well as multilateral institutions that are looking to invest in climate and green financing should be investigated in addition to educating and making them aware of the growing alternative protein sector. Stakeholders highlighted that establishing manufacturing capacity in India will not only be instrumental in catering to the needs of the Indian market but also in meeting global demands for plant-based meat. India can potentially emerge as the export hub for wet and dry TVP. Stakeholders also pointed out the possibility of leveraging the Production-Linked Incentive (PLI) Scheme to obtain investments for manufacturing plant proteins and TVP through extrusion.

The stakeholders concluded by emphasizing the spirit of collaboration and mindset of abundance that will be instrumental in building the alternative protein ecosystem in India.

Conclusions

High-moisture extrusion is the most feasible, scalable, and mature technology available for developing whole-cut meat analogs. Despite the relative maturity of this technology, it is constantly evolving, and the science behind the structuring process that high-moisture extrusion facilitates remains to be fully understood. This leaves much scope for innovation in high-moisture extrusion space to build a variety of end products catering to diverse taste and texture profiles desired by the consumer. While extrusion capacity and capability in India are yet to reach a sizable number, the inherent advantages coming from the biodiversity of crops, volumes of crop produce available, and the ability to operate manufacturing units at low costs can position India as an export hub for TVP production. Making full use of these advantages would require technological innovation and financing to accelerate basic academic research, ingredient development, manufacturing setup, and product development. Hence, mobilizing stakeholders, including policymakers, government institutions, financial institutions, climate-focused multilateral funding institutes, investors, entrepreneurs, corporations, and academic researchers, will be crucial to building momentum for generating structural forces that can push the alternative protein sector forward.

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The participants in our study included -

- Prateek Ghai, Co-founder at BVeg
- Purnachand Upadrashta, Business Head at ICL Food Specialties - India & GCC
- Gaurav Sharma, Chief Advisor at Shandi Global
- Kevin Parikh, Co-founder at Proeon
- Ashish Korde, Co-founder at Proeon
- Sohil Wazir, Chief Commercial Officer at Blue Tribe Foods
- Navneet Singh Deora, Chief Technology Officer at Blue Tribe Foods
- Siddharth Mangharam, Country GM at LIVEKINDLY Collective
- Dejan Djurica, Area Sales Manager, Middle East & Africa, India and East Asia at Bühler Group
- Amit Kher, National Manager, Human Nutrition at Bühler Group
- Christoph Vogel, Head of Market Segment Proteins & Ingredients at Bühler Group
- Chirag Sabunani, Co-founder at Supplant
- Shalom MJ, Regional Sales Manager, India at Coperion K-Tron
- Dr. Gurmeet Singh, Head, Center for Ayurveda, Biology, and Holistic Nutrition at the University of Transdisciplinary Health Sciences and Nutrition (TDU)
- Anuja Ghate, Manager at OrgTree
- Dheeraj Talreja, President at AAK India

» Maria Graefenhahn, Head of the Application Laboratory for Food and Feed at Brabender
» Pratichee Kapoor, Business Development Director (Big Bets) - India, Middle East, Africa at Kerry Ingredients
» Ankit Patel, Director at Liberate Foods
» Natasa Taseski, Process Technologist at Wenger

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1. Introduction section
   a. Classification of meat analogs
      Kyriakopoulou, K., Keppler, J.K. and van der Goot, A.J., 2021. Functionality of ingredients and additives in plant-based meat analogs. Foods, 10(3), p.600.
   b. Overview of techniques for creating meat analogs
      Dekkers, B.L., Boom, R.M. and van der Goot, A.J., 2018. Structuring processes for meat analogs. Trends in Food Science & Technology, 81, pp.25-36.
   c. Others
      McClements, D.J. and Grossmann, L., 2021. The science of plant-based foods: Constructing next-generation meat, fish, milk, and egg analogs. Comprehensive Reviews in Food Science and Food Safety.

2. Raw materials for high-moisture extrusion
   a. Boye, J., Zare, F. and Pletch, A., 2010. Pulse proteins: Processing, characterization, functional properties and applications in food and feed. Food research international, 43(2), pp.414-431.
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3. Plant-based meat - technical and sensory evaluations
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   ii. McClements, D.J., Weiss, J., Kinchla, A.J., Nolden, A.A. and Grossmann, L., 2021. Methods for Testing the Quality Attributes of Plant-Based Foods: Meat-and Processed-Meat Analogs. Foods, 10(2), p.260.

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  b. Impact of process parameters and impact of formulation
   Cornet, S.H., Snel, S.J., Schreuders, F.K., van der Sman, R.G., Beyrer, M. and van der Goot, A.J., 2022. Thermo-mechanical processing of plant proteins using shear cell and high-moisture extrusion cooking. Critical Reviews in Food Science and Nutrition, 62(12), 3264-3280.
  c. Molecular or chemical transformations during extrusion
   Beniwal, A.S., Singh, J., Kaur, L., Hardacre, A. and Singh, H., 2021. Meat analogs: Protein restructuring during thermomechanical processing. Comprehensive Reviews in Food Science and Food Safety, 20(2), pp.1221-1249.
  d. Rheological, phase, and microstructural transformations during extrusion
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