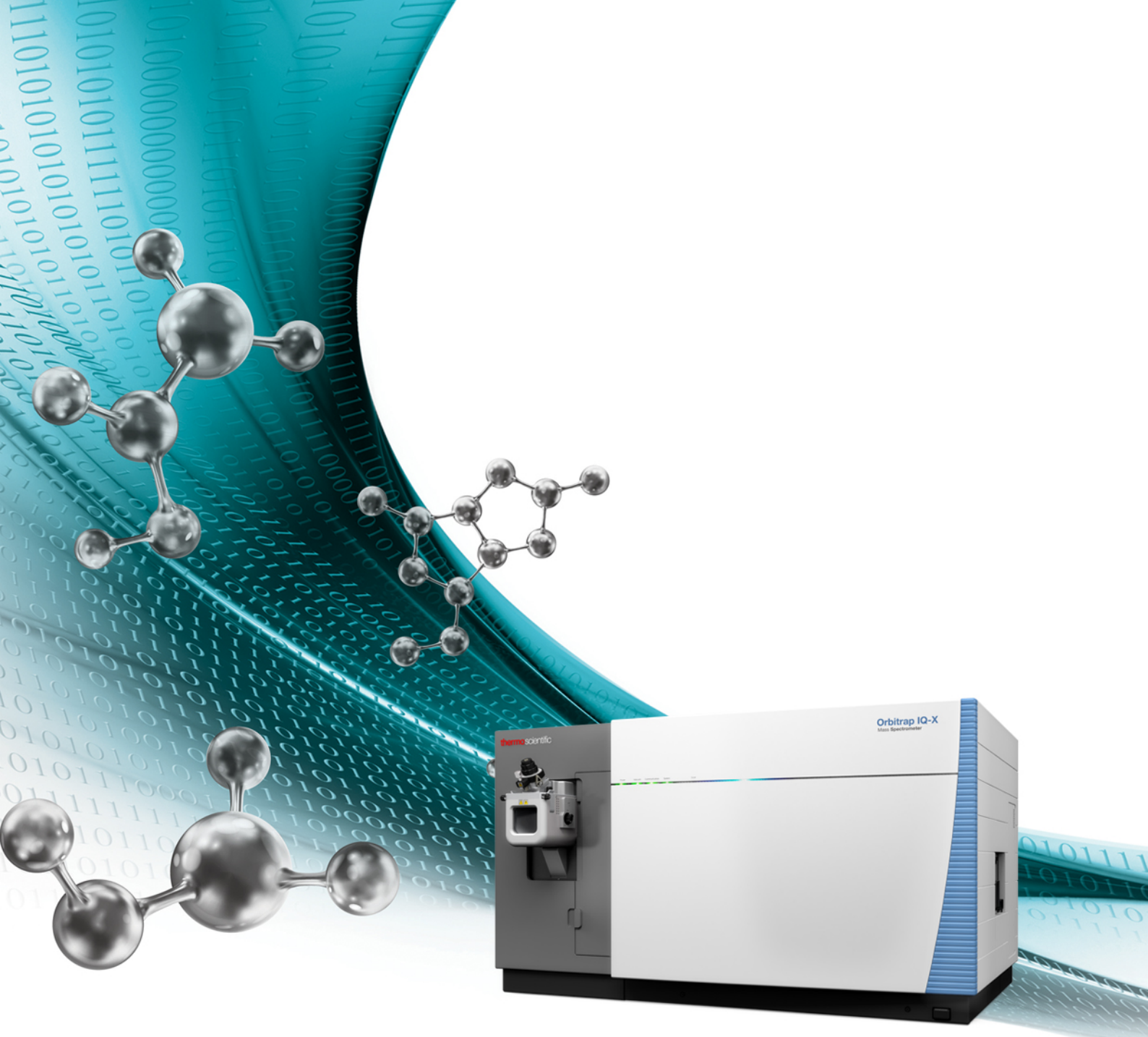

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COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY

Comprehensive REVIEWS in Food Science and Food Safety WILEY

The science of plant-based foods: Constructing next-generation meat, fish, milk, and egg analogs

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Abstract

Consumers are increasingly demanding foods that are more ethical, sustainable and nutritious to improve the health of themselves and the planet. The food industry is currently undergoing a revolution, as both small and large companies pivot toward the creation of a new generation of plant-based products to meet this consumer demand. In particular, there is an emphasis on the production of plant-based foods that mimic those that omnivores are familiar with, such as meat, fish, egg, milk, and their products. The main challenge in this area is to simulate the desirable appearance, texture, flavor, mouthfeel, and functionality of these products using ingredients that are isolated entirely from botanical sources, such as proteins, carbohydrates, and lipids. The molecular, chemical, and physical properties of plant-derived ingredients are usually very different from those of animal-derived ones. It is therefore critical to understand the fundamental properties of plant-derived ingredients and how they can be assembled into structures resembling those found in animal products. This review article provides an overview of the current status of the scientific understanding of plant-based foods and highlights areas where further research is required. In particular, it focuses on the chemical, physical, and functional properties of plant-derived ingredients; the processing operations that can be used to convert these ingredients into food products; and, the science behind the formulation of vegan meat, fish, eggs, and milk alternatives.

KEYWORDS

plant proteins, plant-based egg analogs, plant-based fish analogs, plant-based meat analogs, plant-based milk analogs, sustainability

1 | INTRODUCTION

Throughout the past century or so, the agricultural and food industries have generated a plentiful supply of safe, tasty, convenient, and affordable foods, which has helped to reduce hunger and malnutrition around the globe. It is becoming increasingly clear, however, that modern food production is also harming the health of both people and the planet (Poore & Nemecek, 2018; Springmann et al., 2018; WRI, 2019). The growth in the global population, as well as the increasing affluence of people around the globe, means that greater quantities of protein-rich foods must be produced to satisfy demand (WRI, 2019). Moreover, more and more people are switching to diets that are rich in ultra-processed foods, which often contain high levels of fat, sugar, and salt (McClements, 2019). Changes in the quantity and quality of foods consumed are leading to a rise in diet-related chronic diseases, such as

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obesity, cardiovascular disease, diabetes, cancer, hypertension, and stroke (Willett et al., 2019). One of the main contributors to the adverse effects of modern food production on human and planetary health is the high levels of animal-derived products that are produced and consumed, including meat, fish, milk, egg, and their derivatives (Poore & Nemecek, 2018). Rearing livestock for food, particularly ruminants and to a lesser extent pigs and poultry, is often considerably worse for the environment than producing agricultural crops intended for direct human consumption (such as cereals, legumes, nuts and seeds). In particular, livestock production leads to greater pollution, land use, water use, greenhouse gas generation, and biodiversity loss than plant food production (Willett et al., 2019). However, it is important to note that the magnitude of the environmental impact of switching to a more plant-based diet actually depends on many factors, including the specific types of animal and plant foods that are compared; the region and conditions under which these foods are produced; and the nutritional quality of the proteins obtained (Loveday, 2019). For instance, in some regions of the world it is not possible to economically grow agricultural crops but it is still possible to rear livestock for food, for example, steep or rocky hills. Another reason driving some consumers to reduce their consumption of animal products is related to their ethical concerns about the confining and slaughtering of huge numbers of animals per year (Leiserowitz, Ballew, Rosenthal, & Semaan, 2020; Possidonio, Prada, Graca, & Piazza, 2021). Finally, many consumers are adopting a more plant-based diet because they believe it is healthier than an animal-based one, which is also driving changes in consumer preferences, but this depends on the types of plant- and animal-based foods consumed (Hemler & Hu, 2019; Possidonio et al., 2021). For instance, fresh or minimally processed plant-based foods such as fruits, vegetables, nuts, and whole grains have been linked to improved nutrition, but highly processed ones like many of the current generation of plant-based meat analogs may not provide nutritional benefits because they often contain high levels of saturated fat, salt, and digestible carbohydrates.

The plant-based foods market is one of the fastest growing sectors of the modern food industry. Indeed, the Good Food Institute, a non-profit organization based in Washington, DC, has reported that the sale of plant-based food in all categories grew by nearly 29% from 2017 to 2019, reaching a market value of around $4.98 billion (Crosser, 2020). Moreover, the global plant-based protein market has been predicted to reach over $35 billion by 2024 (R&M, 2020). The plant-based food product category includes a broad range of products designed to mimic animal-based foods, including meat, fish, eggs, milk, and their products (Figure 1). Consumers expect each of these products to have particular physicochemical, functional, and sensory attributes, which leads to specific formulation and production challenges to the plant-based food sector.

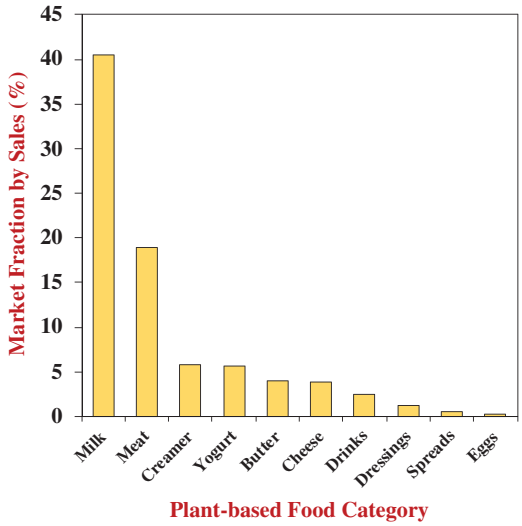

FIGURE 1 Market fraction of retail plant-based food sales by category. The total sales in 2019 were around $5 billion, which is a 29% increase from 2017 to 2019. Data from Crosser (2021)

In summary, there is a rapid transformation occurring within the modern food industry as food manufacturers focus on the creation of plant-based products that are more ethical, sustainable and healthy than the animal-based alternatives. However, these products must also be tasty, convenient, and affordable, otherwise consumers will not purchase them. The production of this new generation of plant-based foods requires a fundamental understanding of the molecular attributes of plant-derived food ingredients, their assembly into nano-, micro- and meso-scale structures, and their contribution to the desirable physicochemical and sensory attributes of the end product. In short, a thorough understanding of structure-function relationships is required. Moreover, the next generation of these foods will have to be carefully designed to have beneficial nutritional profiles, such as micronutrient composition, bioavailability, and digestibility, so that their consumption does not lead to adverse health effects.

2 | PLANT-BASED INGREDIENTS

A number of different plant-derived ingredients can be used to create plant-based foods, with the selection of the most important ones being critical to the development of a successful end product. In this section, a brief overview of some of the most important plant-based ingredients is given, while their main functional properties are discussed in the following section.

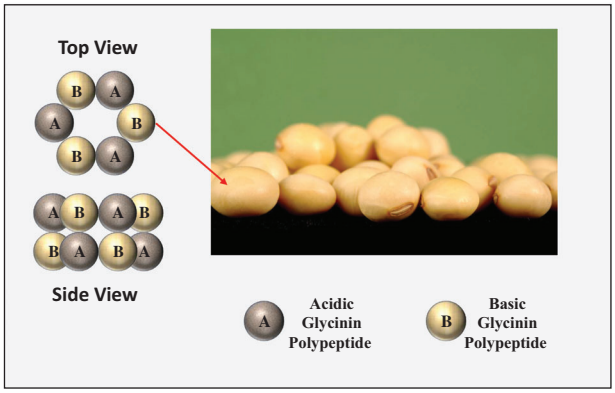

FIGURE 2 The globular proteins in plant materials are often present as multimers that are physically and/or covalently linked together. The functionality of these proteins depends on their denaturation and aggregation state after isolation. Here, the proposed structure of the native form of the 11S-form glycinin molecule is shown, which consists of (a) acidic and (b) basic polypeptide chains. Image of soybeans from CSIRO (Creative Commons 3.0)

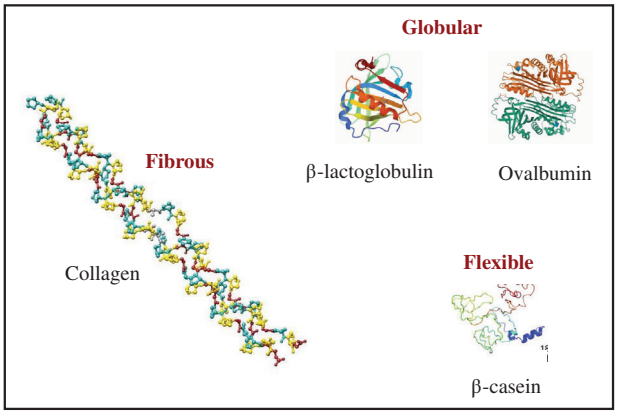

FIGURE 3 The proteins in animal foods come in a variety of different structures, which impacts their functionality. Finding plant-based ingredients that mimic these structures and functionalities is challenging. Protein structures from the Protein Database (NIH): Collagen (1CGD); β-lactoglobulin (3NPO), ovalbumin (1OVA). The structure for β-casein is from: Zhou et al. (2019)

2.1 | Proteins

Proteins are the most important functional ingredient in many plant-based foods, due to their ability to provide unique structuring, texturizing, emulsifying, foaming, fluid holding, and nutritional attributes (Loveday, 2019, 2020). The selection of an appropriate plant protein source is often one of the most critical aspects of creating a high-quality end product. Many of the proteins isolated from botanical sources are relatively large globular storage proteins that are found in nature as part of supramolecular structures (Figure 2). In contrast, many of the proteins from animal-based foods have unique molecular characteristics that determine their desirable quality attributes (Figure 3). For instance, muscle tissues contain a complex hierarchical assembly of bundles of fibrous proteins embedded within connective tissue made from triple helices of collagen (Figure 4), which determines the physical and sensory attributes of meat products (Tornberg, 2013; Tornberg, Andersson, Andersson, & Josell, 2000). The filamentous nature and complex organization of meat proteins are very difficult to simulate using globular plant proteins. In addition, the manufacture and properties of cheeses and yogurts are strongly influenced by the characteristics of the casein molecules and micelles present in cow's milk (Chandan & Kilara, 2013; Fox, Guinee, Cogan, & McSweeney, 2016). Caseins have a highly flexible almost random coil structure, with hydrophobic and hydrophilic patches and numerous phosphate groups (Farrell et al., 2002). The caseins associate with each other to form three-dimensional networks in yogurt and cheese that lead to their unique textural characteristics. These molecular characteristics are again very different from those exhibited by globular plant proteins. A major challenge in this area is therefore to assemble plant proteins into structural arrangements that lead to physicochemical and functional attributes similar to those provided by animal proteins, such as appearance, texture, fluid holding, cookability, and mouthfeel. Some strategies for doing this are reviewed later in this article. Here, a brief description of the key characteristics of different kinds of animal proteins are given (Brady, 2013; Damodaran, 2021), since this knowledge is important for identifying plant-derived alternatives:

- Globular proteins: Globular proteins consist of polypeptide chains that fold into tightly packed structures, almost like a golf ball (Figure 3). The main driving force for protein folding is usually the hydrophobic effect, that is, the reduction in free energy that occurs when the contact area between non-polar groups and water is reduced. Nevertheless, other molecular interactions are also important in holding their structures together, particularly hydrogen bonding (a-helix, b-sheet, and b-turn), electrostatic forces, van der Waals forces, and disulfide bonds. The solubility of globular proteins depends on a number of attributes including molecular weight, their surface hydrophobicity and charge characteristics, typically decreasing with an increase in hydrophobicity and a decrease in charge. Globular proteins are often surface active because they have some non-polar patches on their surfaces that favor their adsorption to oil-water or air-water interfaces, which means they can be used as either emulsifiers or

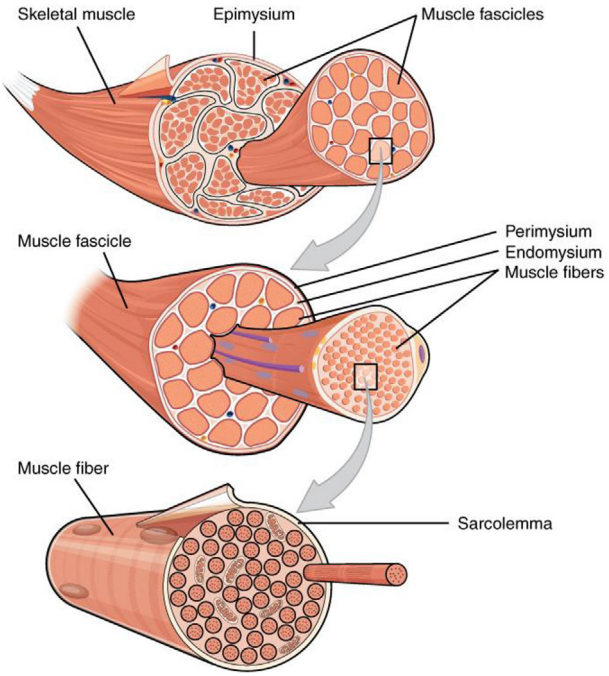

FIGURE 4 The muscles in meat have a complex hierarchical structure that is difficult to mimic using plant proteins. In particular, muscle tissue contains bundles of fibers that are packed into larger fibers. *Attribution*: OpenStax, CC BY 4.0 (https://creativecommons.org/licenses/by/4.0), via Wikimedia Commons

foaming agents. Globular proteins can also be used as gelling agents because they tend to unfold when they are heated, which exposes non-polar and sulfhydryl groups to the surrounding aqueous phase, thereby leading to aggregation through hydrophobic and disulfide bond formation. This kind of functionality is exhibited by the whey proteins in milk or the egg proteins in egg yolk or white. Most plant proteins currently used are globular, but they have different molecular features and functional attributes than the globular proteins found in animals (Tables 1a–d). In nature plant proteins are often part of molecular assemblies containing numerous globular proteins that are either physically or covalently bound together (Figure 2). The functionality of these proteins depends on the degree to which these assemblies are broken down during their isolation and utilization, as well as on their denaturation and aggregation states after release. In addition, the thermal denaturation temperatures and isoelectric points of plant proteins are typically different from those of the animal proteins they are designed to replace (Tables 1a–d), which means they perform differently in many food applications. Consequently, it is often important to select a plant protein that most closely mimics the behavior of the animal proteins in the food product whose properties are being mimicked. The most common plant proteins currently used in plant-based foods include soy, pea, potato, mung bean, and rice proteins, which are all globular proteins (Sha & Xiong, 2020).

- Flexible proteins: There are only a few food proteins that have relatively flexible and fairly disordered structures. The most important animal proteins that demonstrate this kind of behavior are the caseins found in milk (Figure 3). The caseins have polar and non-polar regions on their surfaces, which means they are effective emulsifiers. Moreover, they have numerous anionic phosphate groups, which means that they can be linked together by calcium ions, which is important for their ability to form gels. Gelatin, which is derived from collagen, is a flexible protein at relatively high temperatures, but it forms helical regions when it is cooled, which can then crosslink with each other through hydrogen bonding. As a result, it can form cold-set gels when present first treated to a sufficiently high temperature and then cooled below the coil-helix transition temperature. At present, it has proved very difficult to simulate the properties of caseins and gelatins using plant proteins because they do not have flexible random-coil

TABLE 1a Properties of the major proteins in meat

	Protein % in fraction	M_W (kDa)	pI	T_m (°C)
Connective tissue				
Collagen	50 to 90	300	5 to 8	60 to 70
Muscle				
Hemoglobin	3	67	6.8	67
Myoglobin	1	17 to 18	6.8 to 7.2	79
Actin	13	43	~ 5.2	70 to 80
Myosin	29	480	~ 5.3	40 to 60
Sarcoplasmic	29	20 to 100	Varies	50 to 70

Abbreviations: M_W, molecular weight; pI, isoelectric point; T_m, thermal denaturation temperature. Adapted from Brady (2013), Damodaran et al. (2021), Friess (1998), Park et al. (2012), Purslow (2018), and Toldra (2017)

TABLE 1b Properties of the major proteins found in egg white and yolk

	Protein % in fraction	M_W (kDa)	pI	T_m (°C)
Egg yolk			**6.0**	
Livetins (α, β, γ)	12	33 to 203	4.3 to 7.6 (5.3)	83.3
Phosvitin	7	35	4.0	80.0
HDL	12	Colloidal		
LDL	68	Colloidal	3.5	
Egg white			**4.5**	
Ovalbumin	58	45	4.6	85
Conalbumin	13	80	6.6	63
Ovomucoid	11	28	3.9	70
Ovoglobulins	8	30 to 45	5.5 to 5.8	93
Lysozyme	3.5	14.6	10.7	78
Ovomucin	1.5	210	4.5 to 5.0	—

Abbreviations: M_W, molecular weight; pI, isoelectric point; T_m, thermal denaturation temperature. Here, HDL and LDL refer to high and low density lipoproteins, respectively. Adapted from Brady (2013), Chalamaiah et al. (2017), Chung & Ferrier (1995), and Ternes (1989).

TABLE 1c Properties of the major proteins found in the casein and whey fractions of cow's milk

	Protein % in fraction	M_W (kDa)	pI	T_m (°C)
Casein			**4.6**	—
α_{S1}−casein	39	23.6		—
α_{S2}−casein	10	25.2		—
β−casein	36	24.0		—
γ−casein	3			—
κ−casein	13	19.0		—
Whey			**5.2**	
β−lactoglobulin	51	18.4	5.4	72
α−lactalbumin	19	14.2	4.4	35 and 64*
BSA	6	66.3	4.9	64
Immunoglobulins	12	Range	Range	Range
Lactoferrin	1 to 2	78	8 to 9	70 and 90*

Abbreviations: M_W, molecular weight; pI, isoelectric point; T_m, thermal denaturation temperature. Adapted from Boye & Alli (2000), Brady (2013), Damodaran et al. (2021), and Wang et al. (2019).
*The lower and higher temperatures for α-lactalbumin and lacterferrin are for the apo- (calcium or iron free) and holo- (calcium or iron bound) forms, respectively.

structures. Nevertheless, it may be possible to use plant polysaccharides, either alone or in combination with plant proteins, to simulate some of the unique characteristics of flexible animal proteins, such as the ability to form transparent cold-set gels in the case of gelatin.

- Fibrous proteins: Some important proteins found in animal-based foods have fiber-like characteristics. For instance, collagen is a rigid rod-like molecule that consists of a triple helix of intertwined polypeptide chains (Figure 3), which is found in the connective tissue of animals. This molecule unravels when it is heated above a certain temperature, which leads to a softening of meat products. The actin and myosin molecules present in muscle tissues are packaged into fiber-like structures, which again contribute to the textural attributes of foods. Again, there are only a few economically viable examples of plant proteins that naturally exhibit this kind of fibrous structure (e.g., glutenin) and only a few numbers of mycoproteins that have been exploited for such purposes (Delcour et al., 2012), which means it is challenging to simulate the properties of animal products where fiber-like proteins play a critical role in determining their properties, such as meat and fish. Nevertheless, physicochemical and processing methods can be utilized to coax plant proteins into such structures (see later).

TABLE 1d Properties of the major proteins found in plant sources

	Protein in fraction (%)	M_W (kDa)	pI	T_m (°C)	Comments
Soy					
Globulins					
β-conglycinin (7S)	17 to 24	150 to 200	5	80	Trimer, globular
Glycinin (11S)	36 to 51	300 to 380	4.5	93	Hexamer, globular
Pea					Multimers, globular proteins
Globulins	55 to 80		4.5	75 to 79	
- Legumin (11S)		360			
- Vicilin (7S)		150			
- Convicilin		280			
Albumins	18 to 25				
- Albumin (2S)		50	6.0	110	
Lentil			4.5	120	Multimers, globular proteins
Globulins	51	15 to 92			
Albumins	17	20 to 82			
Legumins	45	14 to 92			
Vicilins	4	20 to 82			
Glutelins	11	17 to 46			
Prolamins	4	17 to 64			
Chickpea			4.5	90	Globular proteins
Globulins	74	15 to 92			
Albumins	16	20 to 82			
Prolamins	0.5	17 to 64			
Lupin			4.5	79 to 101	Multimers, globular proteins
Globulins	75	150 to 216	5.6 to 6.2	103	
Albumins	25		4.3 to 4.6		
Canola		14 to 59	4.5	84 to 102	Multimers, globular proteins
Globulins	60				
Albumins	20				
Glutelins	15 to 20				
Prolamins	2 to 5				
Corn zein			6.4	89	Hydrophobic
α-zein	75 to 85	19 to 24			Packed helices
β-zein	10 to 15	14 to 15			
γ-zein	5 to 10	16 to 27			

Abbreviations: M_W, molecular weight; pI, isoelectric point; T_m, thermal denaturation temperature. Adapted from Asif et al. (2013), Boye et al. (2010), Brady (2013), Loveday (2019), Sousa et al. (1995), Tan et al. (2011), Tandang-Silvas et al. (2011), Tang (2017), and Tanger et al. (2020).

There have been a number of excellent recent reviews on the properties of alternative protein sources for creating plant-based foods, and the reader is referred to these for more details (Loveday, 2019, 2020; Sha & Xiong, 2020). In general, commercial plant-based protein ingredients come in three main forms: flours, concentrates, and isolates. Typically, the protein concentration increases as one moves from flours to isolates, but the fraction of native proteins remaining often decreases. Indeed, one of the major problems in the plant-based food area at present is the lack

of consistent ingredients that have the desired functional attributes. In particular, the native structure of the plant proteins is often destroyed during the isolation process, and the individual globular proteins may be aggregated to various extents, which leads to a reduction in their water-solubility. The water-solubility of plant proteins is important for many food applications, since it is a prerequisite for good functional performance (see later).

A list of animal and plant proteins and some of their important molecular and functional characteristics are included in Tables 1a–d. In general, the molecular characteristics and functional performance of plant-based proteins depend on their biological origin, as well as the extraction and processing conditions used to convert them into food ingredients. Some of the major challenges associated with producing plant protein ingredients with consistent and desirable functional attributes are listed here:

- Off flavors—many plant protein-rich ingredients have undesirable aroma profiles, such as beany, earthy, grassy, musty, and woody (Bangratz & Beller, 2020; Rackis, Sessa, & Honig, 1979). In addition, many plant proteins have unpleasant mouthfeels because they are perceived as astringent or bitter (Bangratz & Beller, 2020). Consequently, these off flavors may have to be removed or masked, or new breeds of plants grown that minimize them.
- Poor solubility—the application of many plant proteins is limited by their poor solubility characteristics. Some plant proteins are soluble in water (albumin), some are soluble in salt solutions (globulin), some are soluble in alcohol solutions (prolamins), and some are soluble in weak alkaline solutions (glutelins) (Osborne, 1924). The percentage of these different proteins varies from species to species. Moreover, the solubility also depends on the state of the protein, for example, whether it is denatured or aggregated. Consequently, it may be important to design the protein isolation and purification procedures to minimize protein denaturation and to ensure the proteins are in an appropriate aggregation state. This would involve controlling factors such as pH, ionic strength, temperature, and solvent conditions.
- Inconsistency—the functional performance of commercial plant protein ingredients often varies from batch to batch due to changes in their composition, denaturation state, aggregation state, or impurities. These variations may be a result of differences in the source material, extraction procedures, processing methods, or storage conditions used. More research is required to identify commercially viable and reproducible processing operations to isolate high-quality and consistent plant protein ingredients.
- Purity—plant protein ingredients often contain impurities (often not specified) that alter their functional performances, such as dietary fibers, starches, other proteins, lipids, phenolic compounds, and minerals. In the future, it will be important to develop protein ingredients with known compositions that only contain the constituents required to create the desired functionality. This may involve producing highly purified ingredients or ingredient blends. For instance, a protein-polysaccharide or protein-mineral combination may perform better than a pure protein for certain applications.

It is clear that more research is needed to develop economic methods of isolating and purifying plant proteins so as to improve their functional performance in plant-based foods.

2.2 | Carbohydrates

Carbohydrates are a class of organic molecules consisting of one or more monosaccharide units linked together by covalent (glycosidic) linkages (Brady, 2013; Huber & BeMiller, 2021). They are commonly found in plants, but less so in animals. Carbohydrates are typically produced in plants through *photosynthesis*, where they are used for a variety of purposes, including as energy sources, signaling molecules, and structural components. Chemically, carbohydrates can be categorized according to their molecular structures, such as the type, number, bonding, and sequence of the monosaccharides they contain (Figure 5). In general, they can be classified as monosaccharides ($n = 1$), disaccharides ($n = 2$), oligosaccharides ($n = 3$ to 10), or polysaccharides ($n > 20$). Many monosaccharides and disaccharides are considered to be sugars because of their inherent sweetness. Plant carbohydrates vary greatly in their molecular, physicochemical, and biological properties, which impacts their functional performance in foods. At the molecular level, they may vary in their molar mass (low to high), structure (linear or branched), and charge (anionic, neutral, or cationic). At the physicochemical and functional level, they may vary in terms of their water-solubility (soluble or insoluble), emulsification properties (good or bad), thickening power (low to high), gelling properties (gelling or non-gelling), and water-holding properties (good or bad) (Williams & Phillips, 2021). On the nutritional and physiological level, they vary in terms of their digestibility (fast, slow, or none) and colonic fermentability (fermentable or non-fermentable). Selection of an appropriate carbohydrate source for utilization in plant-based food products is

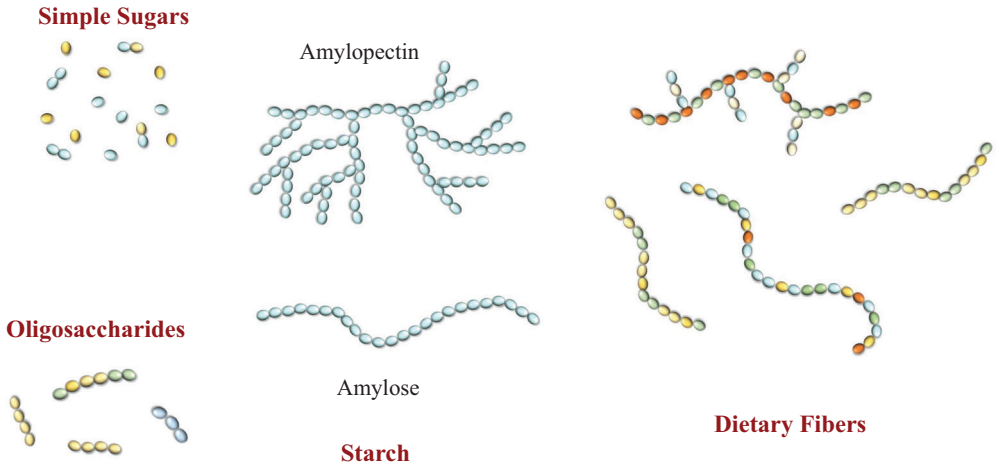

FIGURE 5 The molecular characteristics of plant-based carbohydrates vary depending on their biological origin and isolation/processing, which determines their functional properties in foods and their digestibility/fermentability in the human gut. In nature, these molecules would exist inside complex structures, such as starch granules, inside cells, or between cells

therefore important as it will determine their quality attributes, as well as their nutritional profile.

Monosaccharides (such as glucose or fructose) or disaccharides (such as sucrose) are often used to provide sweetness to plant-based foods (Huber & BeMiller, 2021). Oligosaccharides, such as inulin or oligofructose, may be added as prebiotics to promote the growth of beneficial bacteria in the gut microbiome. Polysaccharides, such as starch, modified starch, methyl cellulose, pectin, guar gum, locust bean gum, pectin, xanthan gum, and gum arabic may be used as thickening, gelling, or emulsifying agents (Williams & Phillips, 2021). Each of these polysaccharides has its own unique physicochemical and functional properties and so it is important to select the most appropriate one for the specific application. For instance, they may be incorporated into plant-based foods to provide desirable textural attributes, fluid holding properties, or health benefits. A number of common carbohydrate sources that can be used in plant-based foods, as well as a summary of their functional properties, is given in Table 2. The functional attributes of polysaccharides, such as thickening, gelling, emulsifying, or fluid-holding properties, are described in more detail in a later section. Like proteins, the functional performance of a particular polysaccharide ingredient depends on its biological origin, extraction procedures, processing methods, and storage conditions.

2.3 | Lipids

The type and concentration of lipids within animal-based products also plays a key role in determining their functionality (Gunstone, 1996). The precise nature of the lipids present depends on the product category, such as meat, fish, egg, or milk. The most common types of lipids found in animal products are triacylglycerols, which consist of three fatty acid molecules covalently attached to a glycerol molecule through ester bonds (Brady, 2013). The fatty acids vary in their chain length, number and position of double bonds, isomeric form (*cis* vs. *trans*), and arrangement on the glycerol molecule (Figure 6). The molecular characteristics of the fatty acids in a triacylglycerol determine the physicochemical and functional properties of lipids. For instance, the change in the solid fat content (SFC) with temperature (Figure 7) depends on the fatty acid profile of the triacylglycerols present. The melting point of lipids tends to increase as the chain length of the fatty acids increases and the degree of unsaturation decreases. It is for this reason that many animal fats, which contain relatively high levels of long-chain saturated fatty acids, such as those found in milk fat or beef lard, tend to be solid-like at ambient temperature (Figure 7). The crystallization and melting properties of fats are critical for providing desirable properties to many animal-based foods, such as the spreadability of butter, the foaming of whipped cream, the meltability of cheese, and the texture of ice cream. Consequently, it is often important to mimic the crystallization properties of animal fats using plant-based alternatives. One of the challenges in this area is that many plant-based fats contain relatively high levels of unsaturated fatty acids (Table 3), which means that they tend to be fluid at room temperature. These fats can be made to be more solid by reducing their degree of unsaturation using hydrogenation processes, but this is often undesirable because it generates trans- or saturated fatty acids, which have been linked to an increased risk of heart disease (Hu, Manson, & Willett, 2001). For this reason, food manufacturers often choose to use natural sources of solid fat, such as coconut oil, because

TABLE 2 Molecular characteristics of some plant-based polysaccharides that can be used as functional ingredients in foods

Name	Source	Main structure type	Major monomer	Function
Agar	Algal	Linear	β-D-galactopyranose	Structuring, thickening, gelling (Ca^{2+}), stabilizing
Alginate	Algal	Linear	β-D-mannuronic Acid	Structuring, thickening, gelling (Ca^{2+}), stabilizing
Carrageenan	Algal	Linear/helical	Sulfated galactan	Structuring, thickening, gelling (K^{+} or Ca^{2+}), stabilizing
Chitosan	Mushroom	Linear	2-amino-2-deoxy-β-D-glucose	Structuring, gelling (polyphosphate)
Guar gum	Seeds	Linear with side chains	D-mannose and D-galactose	Thickening, stabilizing
Gum arabic	Acacia sap	Branched coil domains on protein scaffold	Galactose	Emulsification, stabilizing
Inulin	Plants or bacteria	Linear with occasional branches	β-D-fructose	Prebiotic, thickening
Locust bean gum	Seeds	Linear with side chains	D-mannose and D-galactose	Structuring, thickening, stabilizing
Methyl cellulose	Wood pulp	Linear	Methylated glucose	Thickening, stabilizing, gelling (heat-set)
Pectin(Citrus)	Plant cell walls	Highly branched coil	Glucuronate (backbone)	HM: Structuring, gelling (sugar + heat), stabilizing LM: Structuring, gelling (Ca^{2+}), stabilizing
Pectin (beet)	Sugar beet pulp	Branched coil with protein	Glucuronate (backbone)	Structuring, emulsifying, gelling (sugar + heat; Ca^{2+} or laccase), stabilizing
Starch granules	Corn, potato, rice, wheat	Branched and linear	α-D-glucose	Thickening, gelling, binding, stabilizing
Tara gum	Plant seeds	Linear with side chains	D-mannose and D-galactose	Thickening, stabilizing
Xanthan gum	*Xanthomonas campestris* exudate	Linear/helical (High MW)	β-D-glucose (backbone)	Structuring, thickening, stabilizing

Note: In practice, within each category, commercial polysaccharide ingredients vary in their molecular and physicochemical properties, which influences their functional attributes.

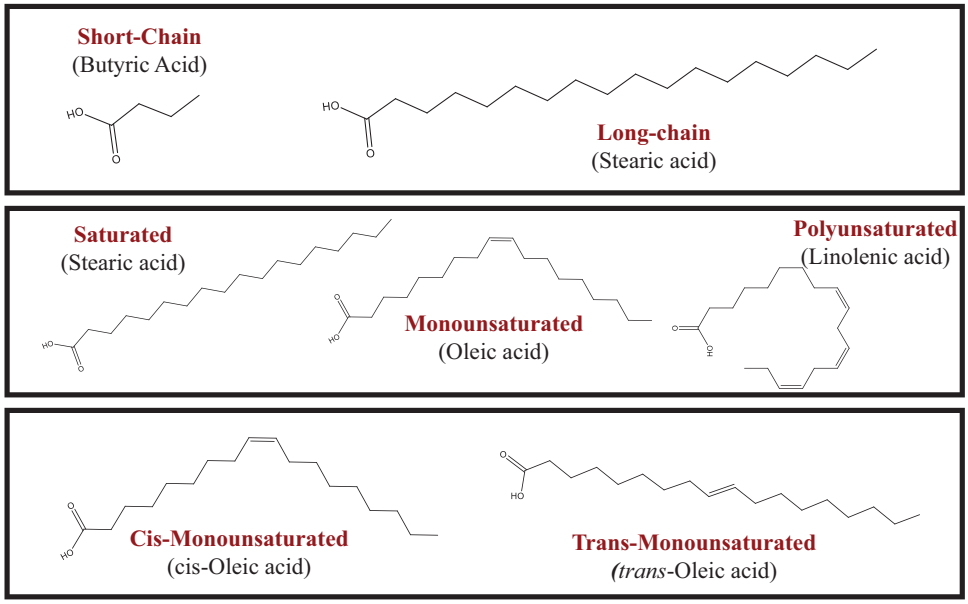

FIGURE 6 The triacylglycerols in animal and plant fats are assembled from fatty acids that vary in the length of their chains, their degree of (unsaturation, and the organization of the double bonds (*cis* vs. *trans*). These molecular features impact their functionality and health impacts in foods. In nature, these molecules would exist inside complex structures, such as oil bodies or adipose tissue. Structures kindly drawn by Yuting Wang

TABLE 3 Comparison of the digestible indispensable amino acid scores (DIAAS) and essential amino acids lacking for different plant and animal sources

Protein source	DIAAS	Limiting amino acid
Corn	36	Lysine
Rice	47	Lysine
Wheat	48	Lysine
Hemp	54	Lysine
Fava bean	55	Methionine + cysteine
Oat	57	Lysine
Rapeseed	67	Lysine
Lupin	68	Methionine + cysteine
Pea	70	Methionine + cysteine
Canola	72	Lysine
Mung bean	86	Leucine
Soy	91	Methionine + cysteine
Potato	100	N/A
Gelatin	2	Tryptophan
Whey	85	Histidine
Casein	117	N/A
Milk	116	N/A
Egg	101	N/A
Pork	117	N/A
Chicken	108	N/A
Beef	112	N/A

Data from Ertl et al. (2016), Han et al. (2020) and Herreman et al. (2020).

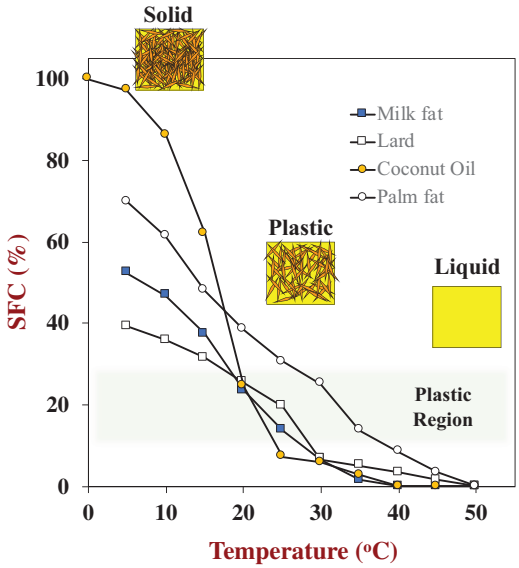

FIGURE 7 The solid fat content (SFC)-temperature profile of edible fats depends on the fatty acid composition, which is determined by their biological origin. Plant-based fats should mimic the SFC profiles of animal-based ones

it has a relatively high solid fat content at ambient temperatures and can therefore provide solid or plastic characteristics (Figure 7). However, coconut oil also contains high levels of saturated fats, albeit medium chain ones (Table 4), which may therefore be detrimental to health (Ludwig, Willett, Volek, & Neuhouser, 2018).

The fatty acid composition of lipids also impacts their nutritional properties and chemical stability (McClements, Decker, & Xiao, 2021). Unsaturated fatty acids, especially ω-3 polyunsaturated ones, are claimed to exhibit various health benefits, particularly the ability to promote heart and brain health (Saini & Keum, 2018; Shahidi & Ambigaipalan, 2018). The fats of terrestrial animals tend to have relatively low levels of these healthy lipids, but those from fatty fish have relatively high levels, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Most plant fats have high levels of monounsaturated or ω-6 polyunsaturated fats. However, some plant-based food oils do contain relatively high levels of alpha-linolenic acid (ALA), which is an ω-3 fatty acid, including flaxseed, walnut, soybean, and canola oils (Rajaram, 2014). For this reason, these oils may be used as a source of ω-3 fatty acids in plant-based foods. However, EPA and DHA typically have more potent health benefits than ALA, and only a small fraction of ingested ALA is converted to EPA and DHA in the human body.

One of the main challenges in fortifying foods with unsaturated fatty acids is that they are prone to lipid oxidation during storage and processing, which leads to the formation of rancid reaction products that consumers find undesirable, and which may be toxic (Arab-Tehrany et al., 2012; McClements et al., 2021; Nogueira, Scolaro, Milne, & Castro, 2019). Special strategies, therefore, need to be employed to overcome these problems: controlling oxygen, temperature, and light exposure by controlling packaging or storage conditions; avoiding contamination with pro-oxidants; adding antioxidants or chelating agents; and food structuring approaches (Jacobsen, 2015; Jacobsen, Sorensen, & Nielsen, 2013; McClements & Decker, 2018). For instance, well-designed colloidal delivery systems can be used to increase the bioavailability of micronutrients in the gastrointestinal tract. The employment of these approaches will be important in the future when creating the next generation of nutritionally fortified plant-based food products.

Some of the most common plant-based lipids used in the formulation of commercial plant-based foods include avocado oil, canola oil, cocoa butter, coconut oil, corn oil, safflower oil, sesame oil, soybean oil, and sunflower oil (Sha & Xiong, 2020). Some of these lipids are partially crystalline at ambient temperature (cocoa butter and coconut oil), which can therefore be used to provide solid-like textures in plant-based foods where this attribute is important.

2.4 | Other additives

Plant-based foods may also contain a variety of other additives to improve their quality attributes, extend their shelf lives, or enhance their nutritional profiles (Sha & Xiong, 2020). A number of the most important ones are highlighted here:

- Colors and flavors: Colorings and flavorings are often incorporated into plant-based foods to enhance their aesthetic appeal. These additives are often needed to make plant-based products look and taste like the animal foods they are designed to simulate. For instance, meat (like beef) is expected to turn from a reddish color in its raw state to a brownish color after it is cooked. Different manufacturers have taken different approaches to simulate the color of meat products. For instance, Impossible Foods uses leghemoglobin, an iron-binding protein originally identified in the roots of soybean plants, to simulate the color of meat. This heme protein undergoes fairly similar color changes as the myoglobin found in real meat. Other companies have used natural colors, such as beet extract, to provide a reddish color to plant-based meat analogs. In the case of plant-based egg analogs, the yellowish color of real eggs can be simulated using natural plant pigments, such as curcumin (turmeric) or carotenoids (from carrots and other

TABLE 4 Fatty acid compositions of common plant and animal fats

Fatty acid	Beef	Pork	Poultry	Salmon	Milk	Egg	Canola	Coconut	Corn	Olive	Palm	Peanut	Sunflower	Soybean
C4:0	–	–	–	–	3	–	–	–	–	–	–	–	–	–
C6:0	–	–	–	–	2	–	–	–	–	–	–	–	–	–
C8:0	–	–	–	–	–	–	–	8	–	–	–	–	–	–
C10:0	–	–	–	–	3	–	–	7	–	–	–	–	–	–
C12:0	–	–	–	–	4	–	–	48	–	–	–	–	–	–
C14:0	3	2	1	3	12	–	–	16	–	–	–	–	–	–
C16:0	27	27	22	11	26	23	4	9	13	10	44	13	7	10
C16:1	11	4	6	5	3	3	–	–	–	–	–	–	–	–
C18:0	7	11	6	4	11	6	2	2	3	2	4	3	3	4
C18:1	48	44	37	25	28	41	56	7	31	78	40	38	14	23
C18:2	2	11	20	5	2	21	26	2	52	7	10	41	75	51
C18:3	–	–	1	5	–	–	10	–	1	1	–	–	–	7
C18:4	–	–	–	2	–	–	–	–	–	–	–	–	–	–
C20:1	–	–	1	–	–	–	–	–	–	–	–	–	–	–
C20:4	–	–	–	7	–	1	–	–	–	–	–	–	–	–
C20:5	–	–	–	5	–	–	–	–	–	–	–	–	–	–
C22:4	–	–	–	2	–	–	–	–	–	–	–	–	–	–
C22:5	–	–	–	7	–	–	–	–	–	–	–	–	–	–
C22:6	–	–	–	17	–	–	–	–	–	–	–	–	–	–
Other	2	1	6	2	6	2	2	1	0	2	2	1	4	5
SFA	37	40	29	18	61	29	6	90	16	12	48	16	10	14
MUFA	59	48	44	30	31	43	56	7	31	78	40	38	14	23
PUFA	2	11	21	50	2	23	36	2	53	8	10	41	75	58

Adapted from Gunstone (2012).

Abbreviations: MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

sources). Often these additives are strongly hydrophobic molecules and so specially designed delivery systems are required to incorporate them into plant-based foods, such as emulsions or nanoemulsions (Zhang, Zhang, & McClements, 2020). Salts, sugars, spices and herbs may also be added to plant-based foods to enhance their flavor profiles and more closely mimic those of animal origin (Sha & Xiong, 2020). Yeast extract is often used to provide a "meaty" flavor in plant-based food analogs (Sha & Xiong, 2020; Watson, 2019) and numerous ingredient manufacturers produce such high quality natural colors and flavors.

- Buffers: Buffering agents, such as phosphates, are often used to control the pH of plant-based foods since this influences their quality attributes and shelf-life. As an example, plant-based creamers may destabilize when added to acidic coffee because the pH becomes close to the isoelectric point of the proteins (Chung, Sher, Rousset, Decker, & McClements, 2017; Chung, Sher, Rousset, & McClements, 2017). In this case, it may be important to maintain a relatively high pH and buffering capacity in the original product to inhibit this problem.
- Cross-linking agents: In semi-solid plant-based foods, such as meats, eggs, and cheeses, it may be necessary to increase the gel strength by adding cross-linking agents (Khalesi, Lu, Nishinari, & Fang, 2020; McKerchar et al., 2019). For instance, enzymes like transglutaminase can be used to form covalent cross-links between plant protein molecules, thereby increasing the mechanical strength of the product so it more closely simulates animal-based structures (Mattice & Marangoni, 2021). Alternatively, cationic mineral ions, like calcium, can be added to aqueous solutions of anionic plant proteins to promote their crosslinking through electrostatic bridging effects (Zhao, Wang, Li, Qin, & Chen, 2017).
- Preservatives: Plant-based foods may also contain plant-based antimicrobials and antioxidants to increase their shelf-life, thereby reducing food waste and improving food quality (Sha & Xiong, 2020). For instance, tocopherols, carotenoids, spices, and herbs can all be used as natural antioxidants, whereas essential oils, curcumin, and polyphenols can be used as natural antimicrobials. It should be noted that botanical preservatives rarely work as well as synthetic ones, and so it is often necessary to use them at relatively high concentrations or in combination.
- Micronutrients and nutraceuticals: It is important that plant-based foods have similar or better nutritional profiles than the animal-based counterparts they are designed to replace, otherwise there may be nutritional deficiencies associated with adopting a more plant-based diet. For this reason, many manufacturers fortify their plant-based food products with micronutrients that might be lacking in a vegetarian or vegan diet, such as vitamin D, w-3 fatty acids, vitamin B12, calcium, iron, or zinc (Tuso, Ismail, Ha, & Bartolotto, 2013). Having said this, there is little evidence that consuming a plant-based diet leads to health problems due to nutritional deficiencies in adult populations in regions where food is abundant, although it has been recommended to supplement the diet with vitamins B12 and D (McDougall & McDougall, 2013). In contrast, individuals consuming a predominantly plant-based diet may suffer diseases related to vitamin or mineral deficiency in regions where foods are not abundant, that is, iron deficiency anemia is widespread in rural India where the diet is mainly cereal-based (Taneja, Rai, & Yadav, 2020). Moreover, there is also some concern that a vegetarian or vegan diet may adversely affect the development of infants due to a lack of adequate levels of vitamins and minerals required for health growth (Biesalski & Kalhoff, 2020). For this reason, it is important to fortify these products with bioavailable forms of essential nutrients that may be missing from a vegetarian or vegan diet. Plant-based foods may also be fortified with bioactive phytochemicals (nutraceuticals) that may improve human health by inhibiting certain diseases, such as carotenoids, curcumin, resveratrol, green tea polyphenols, or ginseng (Calabrese, 2021). Vitamins, minerals, and nutraceuticals often have to be encapsulated within well-designed colloidal delivery systems so that they can be successfully incorporated into food matrices and that they have a suitably high bioavailability (Dima et al., 2020; McClements, 2020b). Alternatively, food matrix effects can be controlled to enhance the bioavailability of these substances (Nair & Augustine, 2018).

3 | INGREDIENT FUNCTIONALITY

The ingredients used to formulate plant-based foods play a variety of roles in determining their physicochemical, sensorial, and gastrointestinal properties. For instance, they may provide desirable optical, textural, mouthfeel, fluid holding, flavor, and stability characteristics. In this section, an overview of some of the most critical functional properties exhibited by plant-based ingredients is given. The main focus will be on biopolymer-based ingredients, such as proteins and polysaccharides, because these are typically the most important ones in many applications. However, a discussion of the crystallization/melting of lipids and the functional attributes of fat droplets is also included, because these play an important role in some plant-based products.

3.1 | Solubility

An important physicochemical attribute of many plant-based ingredients is their solubility in oil, water, or other solvents. In particular, the water-solubility of proteins and polysaccharides from plant origin plays an important role in determining the quality attributes of foods. For instance, the ability to fully dissolve these functional ingredients in water often influences their ability to thicken solutions, form gels, and stabilize emulsions (Phillips & Williams, 2021).

The solubility of biopolymers is governed by their unique molecular features, such as their molar mass, surface hydrophobicity, and electrical characteristics (Guo, Hu, Wang, & Ai, 2017). Typically, their solubility within aqueous solutions decreases as the number of non-polar groups on their surfaces increases because of a strengthening of the hydrophobic attraction between different molecules. This phenomenon accounts for the relatively low water-solubility of hydrophobic proteins, like gliadin and zein (Davidov-Pardo, Joye, & McClements, 2015), as well as the tendency for hydrophobic polysaccharides to self-associate when they are heated, as is the case with methylcellulose (BeMiller, 2019; Murray, 2009). For example, methylcellulose tends to form transparent solutions at low temperatures but cloudy gels at high temperatures due to the formation of hydrophobic crosslinks between the polysaccharide molecules (Arvidson et al., 2013; Spelzini, Rigatusso, Farruggia, & Pico, 2005). Polysaccharides that are able to come close together and form strong intermolecular hydrogen bonds, such as linear cellulose molecules, also have low water-solubilities (Guo et al., 2017).

Water-insoluble proteins like zein have been used to create desirable structural features within plant-based meat products such as fibers or particulates (Mattice & Marangoni, 2020). Similarly, water-insoluble polysaccharides like cellulose have been used as fat replacers, texture modifiers or bulking agents in processed foods. In this case, the crystalline cellulose material is fragmented into microparticles or nanoparticles using chemical or mechanical means, which are then used as functional ingredients in foods (Duan, Huang, Lu, & Zhang, 2018; Khalil et al., 2014).

The ability of ingredients to dissolve in water also depends on their electrical characteristics. A solution of strongly charged proteins or polysaccharides often has a high water-solubility because the strong electrostatic repulsive forces generated by the molecules prevent them from coming close together (Curtis & Lue, 2006). The low water-solubility of many plant-based proteins around their isoelectric points is a result of them losing their electrical charge, so that van der Waals and hydrophobic attractive forces dominate the weak repulsive forces (Gehring, Gigliotti, Tou, Moritz, & Jaczynski, 2010). In systems containing both anionic and cationic substances, the water-solubility can be reduced due to electrostatic attraction between the oppositely charged molecules. An example of this phenomenon is when a cationic protein is mixed with an anionic polysaccharide, leading to the formation of insoluble electrostatic complexes (Weiss, Salminen, Moll, & Schmitt, 2019). The formation of these complexes may be advantageous in some cases because they can lead to desirable structural features, such as fibers or particles. The solubility of large molecules, like most plant-based biopolymers, often increases as their molecular weight decreases because the entropy of mixing becomes more important, thereby favoring their random organization within water (Curtis & Lue, 2006).

The solubility characteristics of biopolymers can be related to their molecular features using theoretical models by assuming they are polymers or colloidal particles dispersed in a solvent, and then calculating the various kinds of forces acting between them, for example, van der Waals, steric, electrostatic, hydrophobic, and entropic interactions (Curtis & Lue, 2006). The biopolymers tend to remain soluble when the repulsive forces dominate the attractive ones but become insoluble when the attractive forces dominate.

3.2 | Binding interactions

Many proteins and polysaccharides used to formulate plant-based foods have complex surface chemistries involving one or more types of polar, non-polar, anionic, and/or cationic groups (Phillips & Williams, 2021). These surface groups may vary in their number and spatial distribution along the biopolymer chains, which impacts their ability to interact with other molecules in their environment (Foegeding & Davis, 2011; Stephen, Phillips, & Williams, 2006). Biopolymers with exposed non-polar regions on their surfaces, such as many plant-based globular proteins, can bind non-polar molecules through attractive hydrophobic interactions. Biopolymers with exposed anionic or cationic regions can bind oppositely charged molecules in their environment as a result of attractive electrostatic interactions (Blackwood, Salter, Dettmar, & Chaplin, 2000). Knowledge of these types of interactions is often useful when designing plant-based food products. For instance, plant-based proteins may bind to aromatic flavor molecules in beverages, thereby altering the overall flavor profile (Guo, He, Wu, Zeng, & Chen, 2019; Wang & Arntfield, 2015). In some cases, this would be desirable because it reduces the level of off-flavors, but in other cases it may be undesirable because it alters the anticipated flavor profile. Mathematical models have

been developed to describe the influence of the molecular characteristics of flavor molecules on their binding to proteins in foods and how this influences the overall flavor profile (Viry, Boom, Avison, Pascu, & Bodnar, 2018).

In plant-based milk and cream analogs, it is important to identify and understand any binding interactions that occur between the fat droplets/oil bodies and other ingredients in the surrounding aqueous phase, as this can impact the stability of the overall system. As an example, the binding of an oppositely charged polysaccharide to the surfaces of protein-coated oil droplets can promote their aggregation through a bridging flocculation mechanism (Dickinson, 2019). Conversely, the complete coating of protein-coated oil droplets with a layer of oppositely charged polysaccharide molecules can protect it from aggregation by increasing the steric and electrostatic repulsion between the droplets (Li & de Vries, 2018; Xu, Sun, & McClements, 2020). Plant protein-coated oil droplets may also be influenced by binding to mineral ions in the aqueous phase. For instance, the binding of cationic calcium ions to the surfaces of anionic proteins or protein-coated oil droplets can reduce the electrostatic repulsion between them, thereby promoting their aggregation (Marquez, Wagner, & Palazolo, 2018). Thus, elucidating, understanding, and controlling the binding interactions in these kinds of products is critical.

3.3 | Fluid-holding capacity

Semi-solid plant-based foods, such as meat, egg, and yogurt analogs, typically contain substantial amounts of fluids, such as pure liquids (e.g., water or oil), solutions (e.g., brine or sugar solutions), or dispersions (e.g., emulsions or suspensions). The presence of these fluids influences the appearance, texture, flavor and stability of these foods. For instance, the retention of fluids in meat-like products plays a critical role in determining their perceived texture and juiciness (Cornet, Snel, Lesschen, van der Goot, & van der Sman, 2021a), whereas the separation of fluids from yogurts is visibly unappealing (Grasso, Alonso-Miravalles, & O'Mahony, 2020). Consequently, it is often important to control the ability of plant-based foods to retain their fluids during storage and food preparation.

Biopolymers are often incorporated into plant-based foods, such as meat, egg, and yogurt analogs, to improve their water holding capacity (WHC), that is, their ability to retain water (or other aqueous-based fluids) within the food structure (Cornet et al., 2021a; Grasso et al., 2020). The presence of this water often plays a vital role in determining the texture and mouthfeel of plant-based food products, such as the juiciness of meat analogs. Consequently, it is important to ensure that the water does not separate from the product. Biopolymers are able to promote water retention by forming a porous 3D-network that holds water through capillary and hydration forces (Blackwood et al., 2000; Stevenson, Dykstra, & Lanier, 2013). The amount of water molecules that bind directly to the surfaces of biopolymer molecules depends on their surface chemistry, as well as on the total surface area exposed. The strength of the capillary forces can be approximated by the Laplace pressure of a porous material (Stevenson et al., 2013):

$$\Delta P = 2\gamma \cos\theta / r \tag{1}$$

In this expression, ΔP represents the capillary pressure, γ represents the surface tension, θ is the water-material contact angle, and r is the pore radius. This equation shows that the capillary pressure increases as the pore size decreases and the surface tension increases.

Physically, the WHC is a measure of a food's ability to retain water in the presence of an external force, such as gravity or an applied stress (such as pressing or centrifugation). An increase in the number and/or decrease in the size of the pores in a porous material tends to lead to an increase in its WHC. Consequently, it is often useful to create a fine uniform porous structure in a food to avoid water loss.

Researchers have attempted to relate the WHC of polymeric porous foods (including meats and plants) to their structural and chemical properties using thermodynamic approaches (van der Sman, 2012, 2013; van der Sman, Paudel, Voda, & Khalloufi, 2013). The mathematical theories resulting from this kind of analysis have highlighted that there are three important contributions that influence the swelling pressure and consequently the WHC of biopolymer-based foods: (i) biopolymer-water mixing; (ii) biopolymer-ion interactions; and (iii) elastic effect contributions resulting from gel deformation. These models can be useful for designing foods with improved fluid holding properties by identifying the importance of key molecular and physicochemical properties (Cornet et al., 2021a; van der Sman et al., 2013). Typically, the ability of a biopolymer matrix to absorb water and swell is reduced as the crosslinking density between the biopolymer molecules is increased because this increases the shear modulus of the gel network, thereby making it more difficult for expansion to occur (Cornet et al., 2021a).

3.4 | Thickening

Plant-based biopolymers (especially polysaccharides) are often used as functional ingredients in fluid or semi-solid plant-based food products to increase the viscosity of the aqueous phase, including milks, creams, fluid eggs,

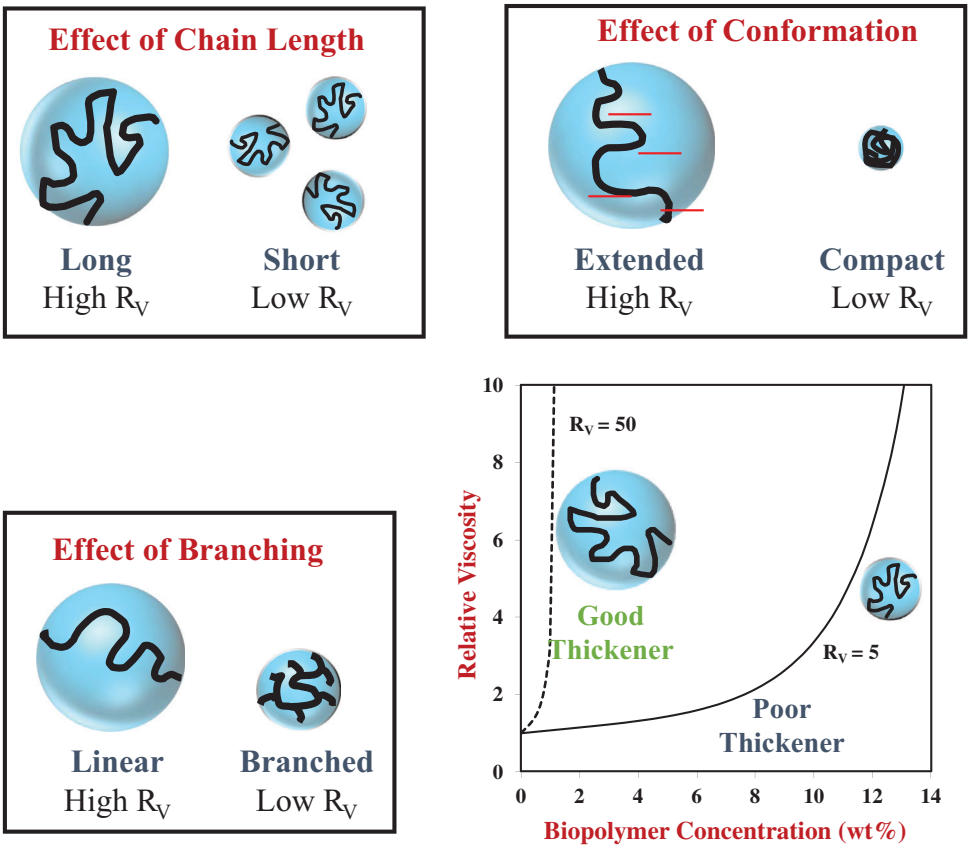

FIGURE 8 The thickening power of biopolymers depends on their molecular characteristics, such as the volume ratio *RV*. Typically, the thickening power increases with increasing molecular weight, increasing extension, and decreasing branching

dressings, and sauces (Williams & Phillips, 2021). These thickening agents may be utilized to generate desirable textural or mouthfeel attributes, or to reduce the rate of gravitational separation of particulate matter (such as fat droplets, oil bodies, plant tissue fragments, herbs, or spices). The ability of a thickening agent to enhance the viscosity of an aqueous solution can be described by its *thickening power*, which is a measure of how much of the ingredient is required to produce a large increase in viscosity. A good thickening agent typically only requires a small amount to effectively increase the viscosity. The thickening power of a biopolymer depends on its volume ratio (R_V), which is the effective volume occupied when it is dissolved in solution (biopolymer molecule + entrapped water) divided by the volume occupied by the biopolymer molecule itself (Bai et al., 2017). The higher the value of R_V, the greater the thickening power of the polysaccharide. In general, R_V increases as the molar mass increases, the degree of branching decreases, and the conformation of the molecule becomes more extended (Figure 8). For this reason, stiff elongated polysaccharides (such as xanthan gum) have a much higher thickening power than compact polysaccharides (such as gum arabic) or proteins (such as pea or soy proteins).

The ability of a biopolymer to thicken an aqueous solution can be modeled using the following equation (Bai et al., 2017):

$$\eta = \eta_1 \left(1 - \frac{\phi_E}{0.57}\right)^{-2} \tag{2}$$

Here, η and η_1 represent the shear viscosities of the biopolymer solution and the solvent, ϕ_E is the *effective volume fraction* of the solution occupied by the biopolymer molecules (including any entrained water). Roughly, the value of ϕ_E is given by the following expression (Bai et al., 2017):

$$\phi_E = \frac{4}{3}\pi r_H^3 \left(\frac{c\,N_A}{M}\right) \tag{3}$$

Here, r_H represents the hydrodynamic radius of the biopolymer molecules in solution, c is the concentration of the biopolymers, N_A is Avogadro's number, and M is the molecular weight of the biopolymer molecules. Predictions made using these equations show that the thickening power of a biopolymer becomes stronger as its effective volume increases (Figure 8).

To a first approximation, the thickening power (TP) is given by the following simple expression, which represents the reciprocal of the biopolymer concentration required to produce a large increase in viscosity (Grundy, McClements, Ballance, & Wilde, 2018):

$$TP = \frac{r_H^3}{21M} \tag{4}$$

In this expression, the units of TP are wt%$^{-1}$ when the molecular weight of the biopolymer is given in kg mol^{-1} and the hydrodynamic radius is given in nanometers. This expression indicates that the thickening power becomes stronger as the hydrodynamic radius of the biopolymer increases (at a fixed molecular weight). In other words, biopolymer molecules that entrap large quantities of water are the most effective at thickening solutions (Figure 8).

Another important attribute of a thickening agent is how the viscosity of the solution changes as the applied shear rate is increased. Most biopolymer-based thickening agents exhibit shear-thinning behavior, that is, the viscosity decreases as the shear rate ($\dot{\gamma}$) increases. This kind of behavior can be described by a power-law model:

$$\eta = K\dot{\gamma}^{n-1} \tag{5}$$

Here, K is the consistency index and n is the power law index. For ideal fluids, where the viscosity does not depend on the applied shear rate, $n = 1$. For shear thinning fluids, $n < 1$, and for shear thickening fluids, $n > 1$. Shear thinning is important because it affects the pourability of fluids, such as plant-based dips, sauces, and dressings. It also influences the perceived mouthfeel of food products. Consequently, it is important to ensure that the thickening agent used provides the required shear-thinning behavior.

It should also be noted that some semi-solid plant-based foods are required to have a yield stress. When a stress below this value is applied to them, they behave like elastic solids but when the applied stress exceeds this value they behave like viscous fluids (Rao, 2007). Products such as dips, sauces, and dressings should have a yield stress. This value should be high enough that the products do not collapse under their own weight after they have been applied to a food, but low enough so that they flow from a container.

3.5 | Gelling

Biopolymers, such as proteins and polysaccharides, are often used as gelling agents to provide desirable textural characteristics to plant-based foods, such as meat, egg, cheese, and yogurt analogs. Many plant-based biopolymers can associate with each other through physical or chemical interactions under appropriate conditions, thereby forming a porous 3D-network of biopolymer chains that traps water inside (Phillips & Williams, 2021). The quality attributes of these biopolymer hydrogels, including their optical, rheological, and fluid holding properties, as well as their sensitivity to environmental conditions (such as temperature, pH, or salt) are governed by the type and concentration of the biopolymers used, as well as the nature of the crosslinks between them. Biopolymer gels can be formed that vary in their appearance (transparent, cloudy, or opaque), texture (soft/hard, rubbery/brittle, fracture properties), setting characteristics (heat-, cold-, ion-, pH-, or enzyme-set), and gastrointestinal fate (digestible/indigestible, fermentable/non-fermentable) (McClements, 2021). Consequently, it is important for formulators to select an appropriate biopolymer or combination of biopolymers to obtain the desired characteristics. Some of the key factors influencing the nature of the gels formed from plant-based proteins and/or polysaccharides are highlighted here.

The overall gel strength (elastic modulus) of a biopolymer gel is determined by the type, concentration, and interactions of the biopolymer molecules within the hydrogel network (Vilgis, 2015). For relatively simple systems, theoretical models are available to link the molecular characteristics of biopolymer gels to their textural attributes (Gabriele, de Cindio, & D'Antona, 2001; Rubinstein, Colby, Dobrynin, & Joanny, 1996). Predictions made by these models indicate that the gel strength increases as the biopolymer concentration, crosslinking density, and bond strength increase (Cornet et al., 2021a).

Many plant-based foods contain particles embedded within a hydrogel matrix, such as fat droplets or protein particles within plant-based meat, fish, egg, yogurt, or cheese analogs. The rheological behavior of these composite systems can also be described using mathematical models (Fu, Feng, Lauke, & Mai, 2008). Predictions made by these models indicate that the concentration, dimensions, and interactions of the embedded particles with the polymer network determine their textural attributes, such as gel strength, stiffness, and toughness. Knowledge of these theoretical models can be beneficial to food formulators because they help to identify the major factors that impact the textural attributes of semi-solid plant-based foods. Having said this, the compositional and structural complexity of most plant-based foods means that only qualitative (rather than quantitative) predictions can be made. A number of the most important attributes of biopolymer gelling agents that need to be considered when selecting them for plant-based food applications are briefly summarized here:

- Final gel strength: The final gel strength (modulus) determines the hardness/softness of the gel, as well as the sensory perception of the gel during mastication (particularly the first bite).
- Gelation temperature: In some applications, the temperature (range) at which a gel forms or melts is important. For instance, a plant-based egg analog should form an opaque gel when it is heated above about 70°C to mimic the cooking properties of real egg, whereas a gelatin analog should form a clear gel when it is cooled below about 35°C.
- Minimum concentration: The lowest biopolymer concentration required to form a gel depends on the molecular structure of the biopolymer molecules. Typically, the biopolymer molecules must form a 3D network that extends throughout the entire volume of the sample. Small spherical molecules like native globular proteins often require much higher concentrations (often >10%) to form gels than large linear molecules like anionic polysaccharides (often <1 wt%).
- Fragmentation properties: The stress or stain where a gel breaks when a force is applied determines its brittleness or rubberiness. Moreover, the way that the gel fractures when it breaks is also important, for example, the size and shape of the fragments formed. The fragmentation properties are particularly important in products like plant-based cheese and meat analogs.

Examples of different kinds of plant-based biopolymers and their gelling properties are highlighted in Tables 1a–d and 2. Many plant-based proteins form gels when they are heated because this causes the globular protein molecules to unfold, thereby exposing non-polar surface groups that can associate with each other through hydrophobic attraction (Mezzenga & Fischer, 2013). Nevertheless, the pH and ionic strength must also be controlled so as to reduce the electrostatic repulsion between the protein molecules.

Alternatively, proteins can be made to form gels by adding crosslinking enzymes such as transglutaminase, which binds different proteins together (McKerchar et al., 2019). Plant-based polysaccharides can be made to form gels through various mechanisms (Table 2). As mentioned before, methylcellulose tends to gel when it is heated due to an increase in the hydrophobic attraction between the methyl groups on the molecules (Spelzini et al., 2005). This phenomenon can be useful in the formulation of plant-based fish and meat analogs where a semi-solid texture is required after cooking. Agar, which is derived from seaweed (red algae), can form clear gels through a thermoreversible mechanism: it gels when cooled and melts when heated (Williams & Phillips, 2021). Alginate, which is obtained from brown seaweed, can form gels at ambient temperatures in the presence of cationic divalent mineral ions, especially calcium (Ca^{2+}) ions (Williams & Phillips, 2021). The cationic ions form salt bridges between anionic carboxyl groups on neighboring alginate molecules. The strength of these gels can be modulated by controlling the calcium concentration, alginate concentration, and alginate type (e.g., M-to-G block ratio, molecular weight). Carrageenan based ingredients, which are extracted from red seaweed, can also be used for their gelling properties (Williams & Phillips, 2021). There are a number of different kinds of carrageenan that differ in their molecular structure and functional properties, with the iota and kappa types been the most widely used in the food industry as gelling agents. Iota carrageenan, which contains two sulfate groups per disaccharide unit, forms gels in the presence of calcium ions. In contrast, kappa carrageenan, which has one sulfate group per disaccharide unit, forms gels in the presence of potassium ions. Seaweed derived ingredients can be produced in a sustainable manner by carefully maintaining and harvesting them in coastal areas, which is a desirable attribute for many plant-based food applications. Reversible heat-setting polysaccharides that form transparent gels have been developed as plant-based alternatives to gelatin (Jaswir et al., 2016). Like gelatin, these polysaccharides gel when they are cooled below a critical temperature but melt when they are heated above another (higher) critical temperature. The main challenges in this area are to obtain gelation and melting temperatures, as well as rheological and mouthfeel properties, that simulate real gelatin.

3.6 | Binders and extenders

Biopolymers are often added to plant-based food products as binders, which are ingredients whose purpose is to hold the product together so it does not collapse (Williams & Phillips, 2021). They can also be used as extenders whose purpose is to add body to the product. Binders and extenders work through a variety of mechanisms, including fluid holding, thickening, gelling, and adhesion. Various kinds of plant proteins and polysaccharides can be used as binders or extenders, including soy-, wheat-, pea-proteins, and corn starches. These ingredients are useful in plant-based foods where a semi-solid texture should be maintained, for example, in uncooked burgers, sausages, or nuggets. Commonly, proteins or polysaccharides are used as binders and extenders. For example, wheat gluten is used to bind extruded protein particles together. Polysaccharides such as pectin, guar gum, carrageenan, cellulose, and methylcellulose are also often employed as binders in meat analog products. The molecular characteristics

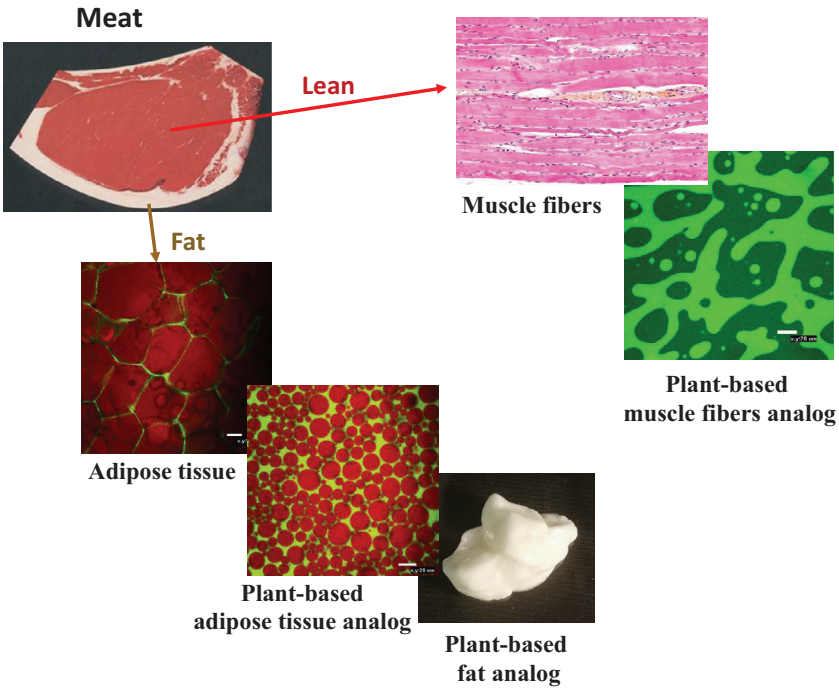

FIGURE 9 Researchers are using soft matter physics approaches to create meat-like structures from plant-derived ingredients, such as proteins, polysaccharides and lipids. Here, the plant-based adipose tissue is a high concentrated oil-in-water emulsion, whereas the plant-based muscle tissue is a phase-separated protein-polysaccharide mixture. The muscle fibers: Nephron, CC BY-SA 3.0 (https://creativecommons.org/licenses/by-sa/3.0), via Wikimedia Commons. Plant-based adipose tissue images kindly supplied by Xiaoyan Hu

and functionality of these ingredients have recently been reviewed elsewhere (Kyriakopoulou, Keppler, & van der Goot, 2021).

3.7 | Emulsification

Emulsifiers are ingredients that can adsorb to oil-water interfaces and stabilize droplets (McClements, Bai, & Chung, 2017). They do this by forming a protective coating around the droplets that provides some mechanical rigidity and/or that generates repulsive forces that help to prevent them from aggregating with each other. The most common repulsive forces are electrostatic and steric repulsion, which depend on the surface charge and thickness of the adsorbed emulsifier layer (McClements, 2015). Plant-based ingredients that can act as emulsifiers are typically surface-active molecules that have some polar and some non-polar regions on their surfaces, or they are small colloidal particles that have appropriate wetting characteristics, that is, they are partly wetted by both oil and water (McClements & Gumus, 2016). Molecular plant-based emulsifiers include some proteins (e.g., from pea, fava bean, lentil, legume, and soy), polysaccharides (e.g., modified starch and gum arabic), phospholipids (e.g., from soy or sunflower), and saponins (e.g., from quillaja or tea) (McClements & Gumus, 2016). Particle plant-based emulsifiers include nanoparticles fabricated from proteins and/or polysaccharides, such as zein, kafirin, and soy protein (Sarkar & Dickinson, 2020; Shi, Feng, Wang, & Adhikari, 2020).

Emulsifiers may be used in plant-based meat analogs to help form simulated adipose tissue. In mammals, adipose tissue consists of large fatty cells (typically tens to hundreds of micrometers) embedded within a network of collagen fibers (Urrutia, Alfonso, & Mendizabal, 2018). This kind of structure can be simulated using plant-based ingredients by preparing a concentrated oil-in-water emulsion containing emulsifier-coated fat droplets, which may be embedded in a biopolymer gel network (Figure 9). These plant-based high internal phase emulsions (HIPEs) are optically opaque semi-solid materials that have physicochemical attributes that mimic some of those of adipose tissue.

Emulsifiers may also be used to facilitate the formation and enhance the stability of the oil droplets in emulsified plant-based foods, such as milk analogs, cream analogs, dressings, and sauces (McClements & Gumus, 2016). In this case, a water phase containing a plant-based emulsifier is homogenized with an oil phase, which leads to the formation of small emulsifier-coated oil droplets (see later). The size and charge on the oil droplets have to be carefully controlled to ensure they are stable to gravitational separation and aggregation. Emulsified lipids play a variety of roles in determining the desirable appearance, texture, mouthfeel, flavor profile, and stability of some plant-based foods (McClements, Newman, & McClements, 2019). For instance, the presence of lipid droplets makes a product look creamy or opaque due to light scattering, as well as increasing the viscosity and creamy mouthfeel by disturbing the fluid flow and lubricating the tongue. Moreover, the lipid droplets may act as a reservoir for lipophilic

aroma molecules, which alters the flavor profile of fatty foods.

3.8 | Foaming

Foaming agents are ingredients that can adsorb to air-water interfaces and facilitate the formation and stabilization of gas bubbles (Amagliani & Schmitt, 2017; Narsimhan & Xiang, 2018). Like emulsifiers, they create a protective coating around the gas bubbles that improves their stability by introducing some mechanical rigidity and/or by generating repulsive forces that can inhibit their aggregation. Foaming agents are useful in products such as plant-based whipped creams, ice cream, or egg analogs, which may contain gas bubbles to provide desirable textural and stability characteristics. A variety of molecular and particulate plant-based foaming agents have been identified that may be suitable for this type of application (Amagliani & Schmitt, 2017; Narsimhan & Xiang, 2018). These are often plant-based proteins or protein nanoparticles, but the functional performance of these proteins is often improved by using them in conjunction with plant-based polysaccharides. In addition, in some cases, the formation of a fat crystal network around the air bubbles can be used to help to stabilize them. For instance, in whipped cream and ice cream partially crystalline milk fat globules aggregate with each other due to partial coalescence, forming a semi-solid shell around the air bubbles. It may therefore be important for the creation of plant-based analogs of these products to identify suitable sources of plant-based fat droplets that can carry out a similar function.

3.9 | Melting/crystallization

The melting and crystallization behavior of plant-derived lipids plays a major role in determining the texture, stability, and mouthfeel of some plant-based foods. In particular, they may be important for creating plant-based meat, cheese, and ice cream analogs that mimic the desirable textural products of the real versions. In this case, the solid fat content (SFC) *versus* temperature profile is important (Figure 7), as well as the morphology, polymorphic state, and interactions of the fat crystals formed (Marangoni et al., 2012; Ramel, Co, Acevedo, & Marangoni, 2016). At a sufficiently high concentration, a 3D-network of small aggregated fat crystals is formed that provides semi-solid characteristics, such as a yield stress and elastic modulus. The gel strength of these fat crystal networks typically increases as the crystal concentration, crosslinking density, and bond strength increases. Ideally, a plant-based lipid phase should match the desirable thermal and textural characteristics of the animal-based one it is designed to replace. This often requires blending different kinds of lipids together to get an appropriate SFC–temperature profile. Commonly, high-melting plant-based lipids like coconut oil or cocoa butter are used for this purpose. Obtaining desirable textural attributes within the lipid phases of plant-based foods typically requires selecting an appropriate lipid source, as well as controlling the cooling rate and temperatures. If this is done correctly, then it will lead to the required SFC–temperature profile, as well as the formation of a network of fat crystals with the required size, shape, polymorphic form, interactions, and mechanical properties.

3.10 | Nutrition

Some additives are used to improve the nutritional profile of plant-based foods. Vitamins and minerals may be added to mimic those found in animal-based foods or to provide micronutrients that might be lacking from a plant-based diet, such as vitamins B_{12}, D, iron, calcium, and zinc. Plant proteins provide a source of essential amino acids to the diet. However, individual plant proteins are typically deficient in one or more essential amino acids, whereas animal proteins are not (Herreman, Nommensen, Pennings, & Laus, 2020). This is highlighted in Table 3, which compares the digestible indispensable amino acid scores (DIAAS) and essential amino acids lacking for different plant and animal sources. Lysine is the main limiting amino acid in cereal proteins, whereas methionine and cysteine are in pulse proteins. It may therefore be possible to overcome this problem by using appropriate blends of cereal and pulse proteins in the same product, or by eating a variety of different sources of protein throughout the day (Herreman et al., 2020). Nevertheless, further research is required to understand how specific protein blends behave within the human gut in different plant-based food matrices, as their nutritional effects will depend on their amino acid profiles, as well as on the rate and extent of their digestion in the upper gastrointestinal tract (Reynaud et al., 2021). As mentioned earlier, lipid sources rich in ω-3 oils (such as flaxseed or algae oils) may also be used to fortify plant-based foods with essential fatty acids, thereby improving their nutritional profile.

3.11 | Gastrointestinal fate

An aspect of ingredient functionality that is often overlooked is their gastrointestinal fate, that is, how they behave within the human gut after ingestion (McClements, 2021). Plant-based ingredients behave

differently during digestion compared to the animal-based ones they are designed to replace (Ogawa et al., 2018). In particular, there are differences in the location, rate, and extent of nutrient digestion and absorption within the gastrointestinal tract (GIT), which influences the pharmacokinetic profiles of nutrients and their metabolites in the bloodstream. Moreover, the composition and digestion rate of a food impacts hormonal and metabolic responses, which impact the susceptibility to diseases such as diabetes, obesity, and heart disease (Xie, Jones, Rayner, & Wu, 2020). Many animal products mainly consist of proteins and fats, with very little carbohydrates, including meat, fish, and eggs (Toldra, 2017). In contrast, many plant-based foods contain digestible and indigestible carbohydrates, such as sugars, starches, and dietary fibers (Figure 5), which behave differently inside the human gut (Mariotti, 2017). A recent in vitro study (INFOGEST) showed that there were appreciable differences in the digestibility profiles of different plant and animal proteins, that is, garden pea, grass pea, soybean lentil, casein, and whey proteins (Santos-Hernandez et al., 2020). In general, the soy proteins were less digested in the gastric and intestinal fluids than the other proteins.

Moreover, the ability of dietary fibers to be fermented by colonic bacteria in the large intestine also impacts the healthiness of plant-based foods (Wilson et al., 2020). The nutritional and health implications of switching from an omnivore to a more plant-based diet are obviously important and will be a critical area for future research (Hemler & Hu, 2019).

3.12 | Other functions

In addition to the properties just described, plant-based ingredients may provide various other desirable properties that are important for mimicking the properties of traditional animal-based foods. For instance, biopolymers may help to suppress the formation of large ice crystals in plant-based frozen meals and ice creams, thereby improving their texture and mouthfeel. They may also inhibit moisture migration, which can help to avoid water loss due to syneresis in food products. This is especially important in plant-based yogurt analogs, where a visible layer of water on top of the product is undesirable.

Other ingredients such as flavors, colors, pH regulators, and preservatives are also important for creating plant-based foods that accurately simulate those of animal origin. The type of flavors and colors depends on the precise nature of the product being simulated. A number of specific examples are given later in the manuscript for particular plant-based foods.

4 | PROCESSING OPERATIONS

Numerous different processing operations can be utilized to assemble plant-based foods, with the specific ones used depending on the nature of the animal food being simulated, such as meat, fish, egg, or milk analogs. Some of them have been used in the food industry for decades, others have been revived, and some have been recently developed. Each unit operation employed must be adjusted for the specific plant-based materials used. For example, plant-derived proteins have different solubility characteristics, denaturation temperatures, and gelling properties than animal-derived ones (Tables 1a–d), which means that different processing windows are required to convert them into end products with similar physicochemical and sensory characteristics.

Commonly plant-based foods are produced by a series of unit operations that are structured into an efficient manufacturing process. The precise process used for a particular food type varies somewhat between food manufacturers, but most plant-based food categories share some common overarching processing operations. In the following section, an overview of the main ones used to structure plant-based foods is given. In general, these operations can be conveniently divided into two main categories depending on the origin of the structuring process: physicochemical and mechanical. In practice, however, both processes are often employed either simultaneously or sequentially in creating structures in plant-based foods.

4.1 | Physicochemical operations

Plant-based ingredients can be used to create structures and physicochemical properties that simulate those in animal products by intelligently controlling the molecular interactions between them. In this section, we highlight some of the most common approaches that are used to do this.

4.1.1 | Phase separation and thermodynamic affinity

Biopolymers interact with each other through various molecular and colloidal forces, as well as entropy effects, that influence their mixing behavior (Fang, 2021). Depending on the nature of the interactions involved, biopolymers exhibit different kinds of behaviors after mixing: miscibility; associative separation; or segregative separation (Figure 10) (McClements, 2006; Tolstoguzov, 1991). Miscibility occurs when the entropy of mixing dominates the

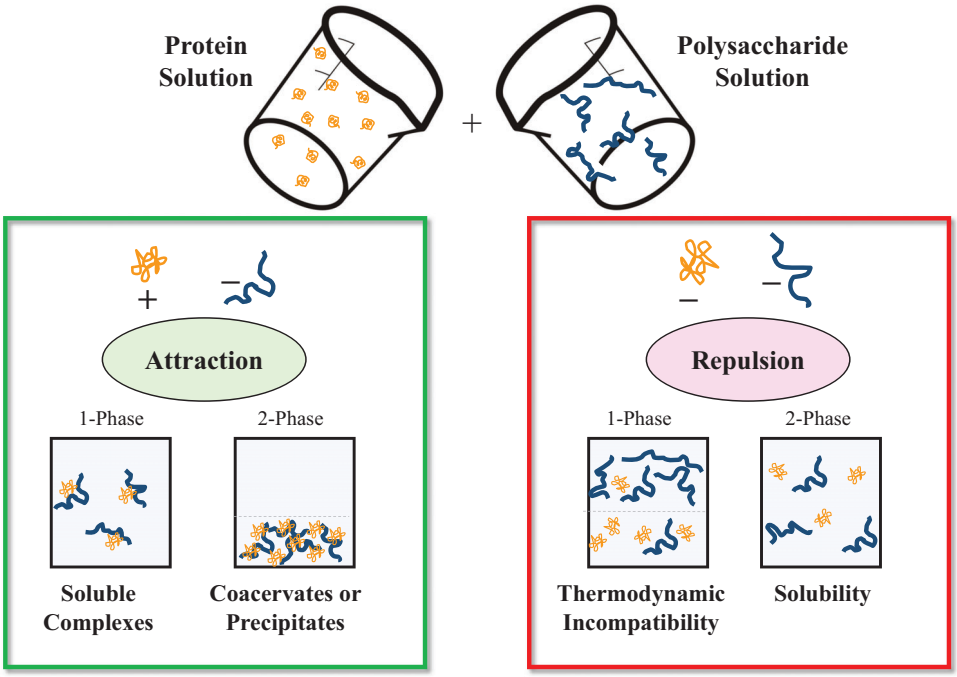

FIGURE 10 A mixture of two plant-based biopolymers, typically a protein and polysaccharide, may form one phase or two phases depending on the sign and strength of the interactions between the biopolymer molecules. Phase separation may occur due to thermodynamic incompatibility or complexation

attractive or repulsive forces acting between the biopolymer molecules. Associative separation occurs when there is a relatively strong attraction between the biopolymers, whereas segregative separation occurs when there is a relatively strong repulsion between them. The tendency for mixtures of biopolymers to separate under certain conditions has been widely used to create meat-like structures in meat analogs (Dekkers, Boom, & van der Goot, 2018a; Kyriakopoulou, Dekkers, & van der Goot, 2019).

The repulsion between the biopolymer chains leads to co-solubility (one-phase) or phase separation (two-phases) depending on system composition (Figure 11). Typically, a one-phase system is formed at low biopolymer concentrations, whereas two-phases are formed at high concentrations. In the case of protein-polysaccharide mixtures, which are often used for the induction of phase separation, one of the phases is protein-rich and the other is polysaccharide-rich. In foods, these thermodynamically incompatible systems may arise under pH conditions where both biopolymers have similar charges. Commonly, a protein and a polysaccharide are used at pH values above the isoelectric point of the protein, so both of the biopolymers are anionic. However, in some cases two proteins with different molecular characteristics may be used.

The separation of the biopolymer mixture into two phases can be utilized to form anisotropic and fibrous structures in plant-based meat analogs (Figure 12). Currently, the segregative separation of biopolymer mixtures, typically a protein and polysaccharide, is the most widely used approach for creating fibrous structures in such systems (Dekkers et al., 2018a). In this operation, a protein and polysaccharide (or two proteins) are mixed together at sufficiently high concentrations to achieve phase

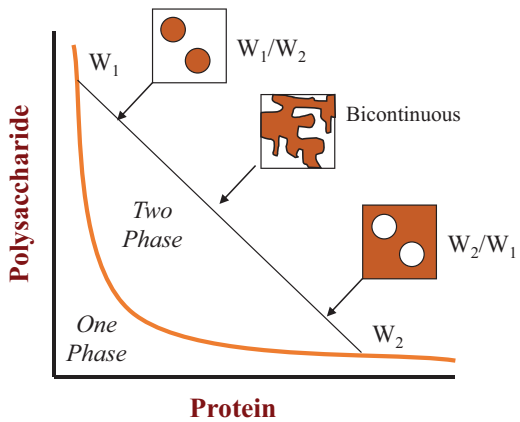

FIGURE 11 Mixed biopolymer systems that separate into two different phases due to thermodynamic incompatibility, which can be described by a phase diagram. In this case, the example of a protein and polysaccharide that are not attracted to each other is given. When the system is stirred it can form a water-in-water emulsion with the continuous and dispersed phases depending on system composition

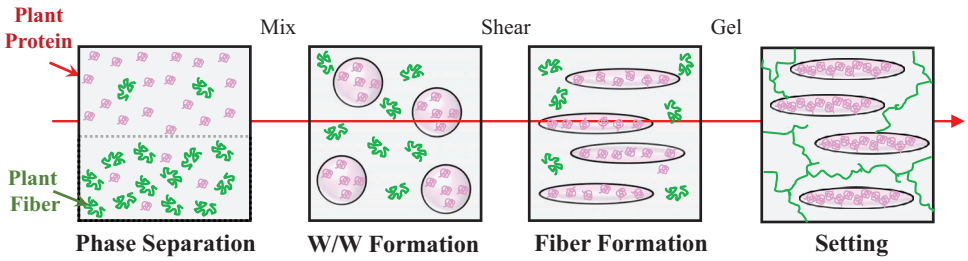

FIGURE 12 Meat-like fibrous structures can be produced from plant proteins and polysaccharides using soft-matter physics approaches: (i) phase separation; (ii) mixing and W/W formation; (iii) shearing and fiber-formation; and (iv) gelling and setting

separation, which leads to the formation of a "water-in-water" (W/W) emulsion. This type of emulsion is characterized by a very low interfacial tension, and thus the internal water phase can easily be deformed and elongated into fiber-like structures by applying low stresses (Dekkers, Hamoen, Boom, & van der Goot, 2018c). The structures formed are then set into place by promoting gelation of the proteins and/or polysaccharides. Studies have shown that this type of anisotropic structure can be formed by simultaneously shearing and heating protein-polysaccharide blends. For example, fibrous structures have been obtained by shearing soy protein isolate and pectin blends together at 130 to 140°C and pH 6 (Dekkers, Nikiforidis, & van der Goot, 2016). Shear-induced structuring is believed to happen in three distinctive phases: (i) blending the two incompatible phases of proteins and polysaccharides, (ii) shearing the phases to cause droplet formation and elongation, (iii) promotion of protein denaturation and aggregation to solidify the structure (Figure 12). During the last step, the elongated polysaccharide-rich droplets are embedded within a protein-rich matrix, resulting in a filamentous texture. It should be noted, however, that the precise origin of structure formation in biopolymer blends is still under debate and new models are being developed to describe this process (Sandoval Murillo, Osen, Hiermaier, & Ganzenmüller, 2019).

The associative separation mechanism has been much less widely used to create structures in plant-based foods, although it also has considerable potential for this purpose. This mechanism is based on structure formation as a result of attractive interactions between biopolymer molecules (Figure 10). The most common interactions utilized are electrostatic attraction between oppositely charged groups. These interactions are typically between a protein and polysaccharide, but they may also involve two proteins with different isoelectric points, or two polysaccharides with different charges (anionic and cationic). In the case of protein-polysaccharide combinations, various structures can be formed around the isoelectric point of the protein, including soluble complexes, complex coacervates, and precipitates (Kayitmazer, Seeman, Minsky, Dubin, & Xu, 2013).

These complexes have also been used as emulsifiers that can improve the resistance of oil-in-water emulsions to environmental stressors, such as pH changes, high ionic strengths, and thermal processing (Evans, Ratcliffe, & Williams, 2013). The formation of a thick charged biopolymer-rich interfacial coating around the oil droplets increases the steric and electrostatic repulsion between them, thereby preventing their aggregation. As a result, stable emulsions can be formed close to or near the isoelectric point of the proteins, which might be useful to formulate stable dairy analogs from plant proteins. For example, it has been shown that caseinate-stabilized emulsions, which are normally destabilized when the pH is lowered to around the isoelectric point of the adsorbed casein (pI ~4.6), remained stable from pH 2 to 7 when dextran sulfate was added (Jourdain, Leser, Schmitt, Michel, & Dickinson, 2008). The dextran sulfate inhibited casein aggregation by forming associative complexes that prevented excessive protein-protein interactions. Although these results were obtained with casein, they can potentially be transferred to plant proteins that are used in dairy alternatives, such as soy and pea protein. Indeed, this approach has recently been used to improve the stability of the protein-coated oil droplets in model rice milks by adding anionic polysaccharides (Xu et al., 2020).

4.1.2 | Protein hydration and solubilization

Protein hydration and solubilization are critical for the production of most plant-based foods, especially meat, fish, milk, and egg alternatives. Protein hydration involves the interaction of water molecules with the protein molecules at various levels. Practically, the solubility of a protein is defined as the fraction that is not separated by centrifugal forces or filtration processes under specified solution conditions, for example, pH, ionic strength, and temperature (Damodaran, 2021). Historically, plant proteins have been

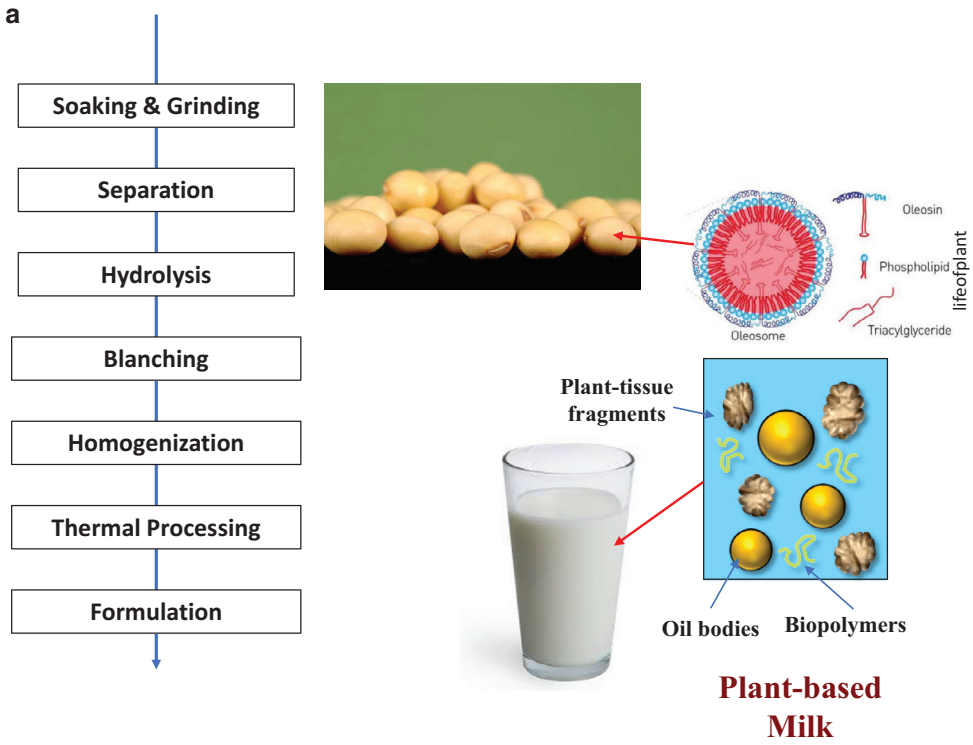

FIGURE 13 Some commonly used processing operations that are utilized to produce plant-based milks. These processes need not be carried out in the order shown. Image of soybeans from CSIRO (Creative Commons 3.0)

characterized according to their solubility under different solution conditions (Loveday, 2019):

- Albumins: Water soluble; aggregate and precipitate upon heating
- Globulins: Water insoluble; soluble in dilute salt solutions (e.g., 100 mM NaCl)
- Prolamins: Water insoluble; soluble in aqueous ethanol solutions (i.e., 70% to 80%); heat resistant
- Glutelins: Water insoluble; soluble in dilute alkaline solutions (e.g., 100 mM NaOH).

Plant protein fractions that are mostly water-insoluble (glutelin and prolamins) are still able to be hydrated and bind water. For example, wheat glutenin was reported to have a low water-solubility at pH 6 (33%) but was still able to hold 5 g of H_2O per g protein under these conditions (Majzoobi, Abedi, Farahnaky, & Aminlari, 2012). In contrast, other plant protein fractions (albumins) have a much higher water-solubility because the repulsive forces acting between them dominate the attractive forces under the prevailing solution conditions. These proteins tend to have less hydrophobic groups on their surfaces, as well as stronger electrical surface potentials.

Knowledge of protein hydration and solubility interactions is important for formulating and processing different classes of plant-based foods. During meat analog processing, the proteins are first hydrated and solubilized before they are texturized into the final structure as this facilitates protein denaturation, aggregation, alignment, and water holding. Most meat analogs are currently produced from protein powders such as soy or pea concentrates or isolates that are rich in globulins, which means that salts often have to be added to increase their solubility and promote their functionality (Dekkers, Emin, Boom, & van der Goot, 2018b). It should be noted, however, that high salt contents can lead to an adverse taste or undesirable health effects.

Production of plant-based milk analogs involves soaking of the raw material (mostly seeds) in water to weaken the cell structure and enable swelling, as well as to facilitate protein hydration and solubilization (Figure 13). After grinding, the solubilized material is obtained by a filtration or centrifugation step (Aydar, Tutuncu, & Ozcelik, 2020). Thus, the final solubilized protein composition depends on the solution (pH, ionic strength, and temperature) and separation conditions (molecular weight cut off or centrifugal force) used. Globulins are the major protein fraction in many plant-based materials used to formulate milk analogs, especially in pea and soy materials. Many of these proteins are therefore not fully soluble at the low salt concentrations found in plant-based milk analogs (Chen et al., 2019). To minimize protein sedimentation and to achieve a stable emulsion, milk analogs are homogenized before

packaging (see below) and texture modifiers (such as gellan gum) are often added to increase the viscosity of the continuous phase, which minimizes gravitational separation. This is one of the main reasons that many plant-based milk analogs are packaged in opaque containers and have the instruction "shake well before use" on their labels. Further research is therefore needed to improve the solubility characteristics of plant protein ingredients, which may be achieved by developing more effective extraction and fractionation processes.

4.1.3 | Setting

As highlighted previously, some plant-derived ingredients, particularly proteins, polysaccharides, and lipids, are able to solidify plant-based foods by forming 3D networks that extend throughout the material. These gels provide solid-like characteristics to the final products, which are desirable in plant-based foods such as meat, fish, cheese, and yogurt analogs. Solid-like textures can be formed through a variety of molecular mechanisms depending on the ingredients used:

- Crystallization/melting—a liquid oil crystallizes when it is cooled sufficiently below its melting point, which leads to the formation of a network of fat crystals, provided there are sufficient fat crystals present;
- Heat gelation—some biopolymers form gels when heated because of an increase in the attractive forces between them at elevated temperatures. Many globular proteins derived from plants unfold when heated above their thermal denaturation temperatures, thereby exposing reactive non-polar and sulfhydryl groups, which promotes their aggregation through hydrophobic and disulfide bonds. Starch granules form gels when they are heated in an aqueous food matrix because they absorb water and swell, causing them to become tightly packed together. These kinds of biopolymers can be used to simulate the heat-set behavior of animal proteins, such as those in egg or milk.
- Cold gelation—some biopolymers form gels when cooled due to an increase in the attractive forces between them at low temperatures. For instance, a number of polysaccharides have random coil structures at high temperatures, but form helices when cooled below a critical temperature. These helices then form crosslinks through hydrogen bonding, leading to the formation of a 3D network of biopolymer molecules that provides mechanical strength. Agar, carrageenan, and starch are examples of this kind of cold-setting gelling agent. These biopolymers may be able to simulate the cold-set behavior of animal proteins such as gelatin.
- Ionic gelation—some charged biopolymers form gels when oppositely charged mineral ions are added through electrostatic screening or bridging mechanisms. For instance, pectin, alginate, carrageenan and anionic proteins (pH > pI) can be crosslinked with calcium ions. Heat-denatured globular proteins, such as soy proteins, can also be gelled by adding salts since they screen the electrostatic repulsion between them.
- Enzymatic gelation—some biopolymers can form gels when specific enzymes are added to crosslink them, most notable proteins by transglutaminase. These enzymes can therefore be used to improve the gel strength of products such as meat, fish or egg analogs.
- pH gelation—some biopolymers form gels when the pH is changed, as this reduces the electrostatic repulsion or increases the electrostatic attraction between them. For instance, many proteins gel near their isoelectric points, whereas mixtures of proteins and ionic polysaccharides gel under pH conditions where they have opposite charges. This type of gelation mechanism is important in the production of cheese and yogurt analogs designed to mimic the tendency for casein molecules to associate and form gels when they are acidified to pH values near their isoelectric point.

In general, it is therefore important to understand the nature of the different kinds of plant-derived ingredients within a plant-based food formulation and design the processing operations to control their structuring and gelation behavior to produce the desired textural attributes in the final product. In particular, it is important to understand factors such as critical temperatures (melting, crystallization, denaturation, and gelling temperatures), pH values, salt levels, and enzyme concentrations.

4.2 | Mechanical operations

Various kinds of mechanical operations are employed in the manufacture of plant-based food products, with the ones used depending on the nature of the product. In this section, a brief overview of some of the most important mechanical operations used in this sector is given.

4.2.1 | High-pressure homogenization

Homogenization is a unit operation that reduces the size of particles in suspensions and emulsions. Particle size reduction is often used to reduce creaming, sedimentation, or aggregation processes, thereby improving the uniformity and shelf-life of the end product (Lopez, Cauty, & Guyomarc'h, 2015b). It may also be used to increase the

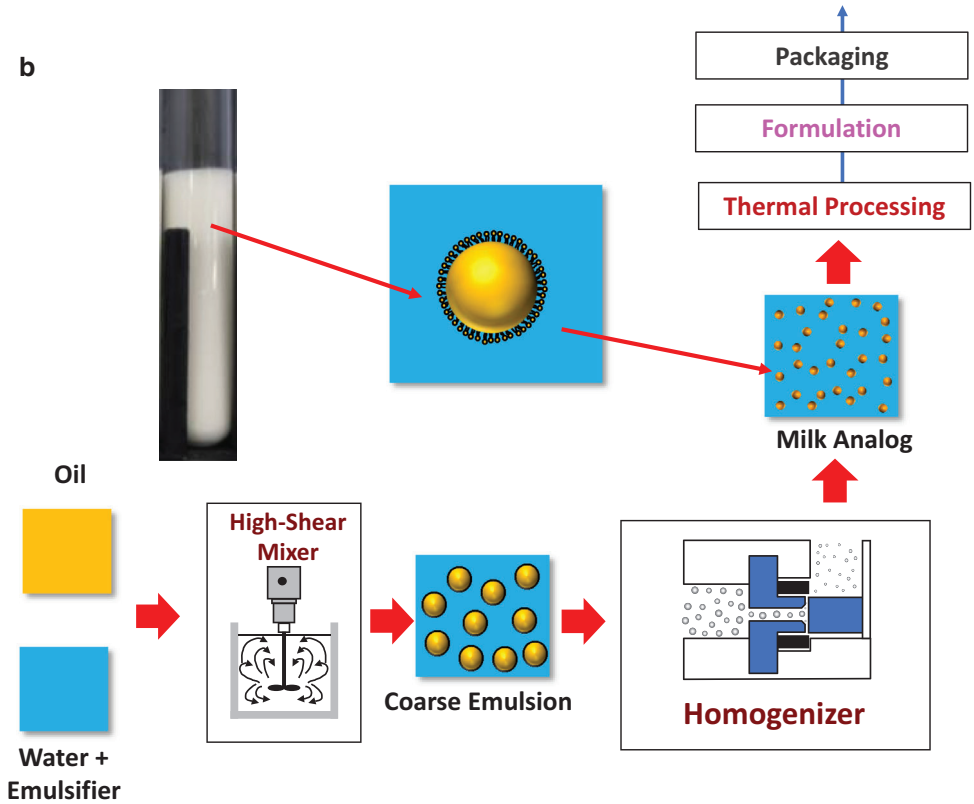

FIGURE 14 Plant-based milks can also be produced by homogenizing plant-based oil and emulsifiers with water. Typically, a coarse emulsion is formed first, which is then passed through a homogenizer

dispersibility and bioavailability of bioactive lipids, since it promotes their digestion and solubilization within the gastrointestinal tract (McClements, 2020b). Different types of machinery can be used to achieve particle size reduction, including high-shear mixers, colloid mills, high-pressure valve homogenizers (HPVHs), microfluidizers, and sonicators (McClements, 2015). HPVHs are currently the most widely used method for preparing low viscosity oil-in-water (O/W) emulsions containing small oil droplets. This type of emulsion can act as the basis for forming plant-based milk analogs (see later). HPVHs consist of two main parts: (i) a high-pressure piston pump driven by an electrical motor; and (ii) one or more valves with an orifice diameter ranging from a few μm to several hundred μm (Figure 14). Initially, a pre-emulsified coarse emulsion is prepared, introduced into HPVH, and then pumped through the valve under high pressure. This process generates intense shear, elongation, and cavitation forces within the fluids that cause the droplets to become stretched and disrupted. The main parameters determining the size of the droplets produced are the valve geometry, the viscosities of the dispersed and continuous phases, the interfacial tension, the energy density, and emulsifier characteristics (Hakansson, 2019). For HPVHs, the energy density E_v (J m^{-3}) is the pressure difference (Δp) across the valve. Typically, the mean droplet diameter decreases with increasing energy density, number of passes, and emulsifier concentration. Consequently, these parameters should be optimized when producing plant-based milk alternatives using high-pressure homogenizers.

High-pressure homogenization has been used to produce plant-based milk analogs from a variety of plant-derived ingredients. A lentil-based milk analog was produced from water, lentil protein isolate (3.3%), and sunflower oil (3.3%) by blending them together using a high shear mixer and then passing them through a HPVH at 180 bar (Jeske, Bez, Arendt, & Zannini, 2019). The milk analog produced had a relatively small mean droplet diameter (<500 μm) and remained stable to separation for over 21 days. A hemp-based milk analog has been produced from whole hemp seeds by soaking, grinding, filtrating, and then homogenizing at 600 bar (Wang, Jiang, & Xiong, 2018). In this case, homogenization reduced the mean droplet diameter from around 5 to 2 μm.

In conclusion, HPVH is already an established operation in the dairy processing industry, which is increasingly being used in the production of plant-based milk alternatives. This is advantageous since it means that the traditional dairy industry can repurpose much of its existing equipment to pivot toward the production of plant-based

foods. Finally, it should be noted that microfluidization and sonication can also be used for this purpose for some applications.

4.2.2 | Extrusion

Ideally, the food industry would like to identify naturally occurring fibrous protein-rich materials to create meat-like structures. However, only mycelia from fungi (mycoproteins) and gluten proteins have so far been identified and exploited for this purpose. For this reason, mechanical processing operations have been developed to convert globular plant proteins into fibrous materials, with extrusion being the most prominent one (Dekkers et al., 2018a). Indeed, plant-based foods based on extruded plant proteins are already found in a variety of product categories, such as patties, nuggets, and sausages.

Extruders are composed of three main parts: (i) an electrical motor to drive the screw(s); (ii) one or two-segmented screws embedded within a temperature-controlled barrel; and (iii) a die to shape the material. For the production of meat analogs a twin screw-extruder is employed with both screws rotating in the same direction (co-rotating) to ensure a high mixing efficiency and a high output (up to 500 kg h^{-1} with current technology) (Caporgno et al., 2020). The barrel and the screw are often divided into a minimum of three sections: (i) a feeding zone consisting of conveying elements operated at $T = 25°C$; (ii) a mixing zone that consists of a set of kneading and paddle blocks operated at $T < 100°C$; and (iii) a final stage (just before the die), which acts as a melting zone with conveying and kneading/reverse elements to increase the mechanical energy input operating at $T > 100°C$ (Osen, Toelstede, Wild, Eisner, & Schweiggert-Weisz, 2014). As the materials move through these different sections, plant proteins and other ingredients are first mixed and hydrated, and then the proteins are denatured and aggregated (Chen, Wei, & Zhang, 2011).

After the barrel section, the protein is fed into the die section. Low-moisture texturized vegetable proteins (TVPs) are typically produced at relatively low moisture contents (<50%) using a short shaping die, often a hole nozzle. The main structuring principle in the die is the pressure drop at the die exit from around 20 to 100 bar to ambient pressure, which causes the hot protein dispersion to expand through flash evaporation. Evaporation causes the denatured protein molecules to become aligned and the structure formed is solidified through the sudden drop in temperature and water content (<20%), which allows the formation of new bonds between the protein molecules. To enhance the shelf life, the TVP is subjected to a final drying step, which enables the product to be stored at room temperature.

In contrast, high moisture TVPs are obtained by mixing the same raw materials at a moisture content of 50% to 70% and attaching a cooling die at the end of the extruder barrel. This process is usually referred to as 'high moisture extrusion' or 'wet extrusion'. The die has a rectangular slit shape, but new die shapes are emerging that also use cylindrical forms to increase the output (Buhler, 2020). In this case, the protein dispersion is forced into the die at low pressures (<20 bar) and the die is cooled by temperature-controlled water at 20 to 80°C (Osen et al., 2014; Pietsch, Emin, & Schuchmann, 2017). In the die, the protein dispersion forms new intra- and intermolecular bonds and slowly cools down without expansion while it gets pushed through the slit exit. The anisotropic structure is formed throughout the die possibly by several mechanisms. Current theories suggest that the fibrous structure is either formed because the laminar flow results in velocity and temperature gradients that create a layered structure or by phase separation mechanisms (Cornet et al., 2021b).

The most important process parameter during extrusion is the specific mechanical energy input (SME) that describes the energy input per unit mass (J kg^{-1}) and is influenced by several variables, including extruder design (such as screw assembly and die design), processing parameters (such as temperature, pressure, and shear rate), and system composition (such as water content and biopolymer type) (Kantrong, Charunuch, Limsangouan, & Pengpinit, 2018; Samard, Gu, & Ryu, 2019). The SME can be calculated using the following equation (Pietsch et al., 2017):

$$\mathrm{SME} = \frac{P - P^0}{\dot{m}} = \frac{\frac{n}{n_{\max}} \mathrm{x} \frac{M^{\mathrm{d}} - M^{\mathrm{d,empty}}}{100}}{\dot{m}} = P_{\max} \left(\mathrm{J\,kg^{-1}}\right) \tag{6}$$

Here, n and $n_{\max}$ are the actual and maximum screw speed (s^{-1}), M_d and $M_{d,empty}$ are the actual and idle torque (Nm), $\dot{m}$ is the total mass flow (kg h^{-1}), and P_{max} and P_0 are the maximum and idle engine power (W). The SME is helpful to compare the effect of different treatment conditions on the raw materials or to design an extrusion process including scale-up in which the SME should be kept constant (Redl, Morel, Bonicel, Vergnes, & Guilbert, 1999).

4.2.3 | Shear cell technology

The shear cell technology was originally developed in the early 2000s at Wageningen University and Research (WUR). Recently, it has been adopted commercially to produce plant-based meat analogs by a start-up

company known as Rival Foods (https://rival-foods.com). This method was originally shown to produce fiber-like structures when milk proteins were sheared in a cone-in-cone setup in the presence of transglutaminase (Manski, van der Goot, & Boom, 2007). Based on these findings, the shear cell technology was adopted for the structuring of plant proteins and successful fibrous structure formation was reported for soy and pea protein blends (Schreuders et al., 2019b). The main advantages of shear cells compared to extrusion technologies are that the shear conditions (shear rate and temperature) can be precisely tuned to create different structures (Krintiras, Gadea Diaz, van der Goot, Stankiewicz, & Stefanidis, 2016). Additionally, the shear cell enables the production of meat/fish analogs with an increased thickness to more closely resemble whole-cuts (Krintiras et al., 2016). However, since it is a batch process that involves a separate pre-mixing step, the production capacity is currently limited.

Two types of shear cell designs have been reported. The first system includes a conical cone-in-cone device, which consists of a lower rotatory cone that is heated and an upper stationary cone that is lowered to achieve a sealed gap in which the protein blends are sheared. Typical processing conditions are shear rates of 0 to 100 rpm and temperatures up to 140°C for several minutes (Grabowska et al., 2016). The second system includes a cylinder-in-cylinder design, which consists of a heated stationary outer cylinder with a lid and a heated inner cylinder that is rotated *via* a drive shaft (Krintiras et al., 2016). The sample is pre-mixed and inserted in the gap between the outer and inner cylinders, which acts as a shearing zone. As an example, processing parameters of 20 rpm, 120°C for 30 min have been used to obtain fibrous structures using a soy protein blend (Krintiras et al., 2016).

Both designs rely on shearing the protein blend under well-defined shear conditions. The shear rate can be calculated according to the following equation for Newtonian fluids and narrow gaps using cone-in-cone designs:

$$\dot{\gamma} = \frac{\omega}{\theta} \left(\mathrm{s}^{-1}\right) \tag{7}$$

Here, ω is the rotor speed (s^{-1}) and θ is the angle of the shearing zone (Peighambardoust, van der Goot, Hamer, & Boom, 2004). For Couette designs, the following equation can be used:

$$\dot{\gamma} = \frac{2 R_i \pi \mathrm{rpm}}{60\, h} \left(\mathrm{s}^{-1}\right) \tag{8}$$

Here, R_i is the radius of the inner cylinder and h is the gap distance between the rotating and stationary cylinders (Krintiras, Göbel, van der Goot, & Stefanidis, 2015). However, more sophisticated models have been used to predict the shear rate for wider gaps and Newtonian liquids (Michels, van der Goot, Norsker, & Wijffels, 2010) as well as for non-Newtonian liquids (Krintiras et al., 2016). Even so, the structuring principle remains the same for all of these designs. The protein blend is first denatured by the influence of heat, and then fibrous structures are formed due to the velocity profile arising from the velocity difference across the gap between the rotatory and stationary elements (Krintiras et al., 2016). The nature of the fibrous structures formed depends on the ingredients and processing operations used. Thermodynamic incompatibility between biopolymer blends, as well as spinodal-phase separations into protein-rich and water-rich phases, are currently being discussed as the underlying driver for the formation of these anisotropic structures (Cornet et al., 2021b).

5 | FORMATION OF PLANT-BASED FOODS

Plant-based ingredients are being used to create a wide variety of food products to replace animal-based ones (such as meat, fish, eggs, and milk) or that normally require animal ingredients as key components (such as cheese, dressings, sauces, spreads, and yogurts). Some of the most common plant-based foods that are designed to replace animal-based ones are highlighted in Figure 1. In the remainder of this paper, we focus on describing the science behind the formulation of plant-based alternatives to meat, fish, eggs, and milk. For each food category, we begin by explaining the properties of the conventional animal-based product, since this is critical for understanding how to formulate high-quality plant-based alternatives. We then focus on how plant-based ingredients can be used to assemble products with the required physicochemical, functional, and sensory attributes. In addition, some of the key limitations in creating these products are highlighted.

5.1 | Meat and fish

The successful commercial introduction of plant-based meat analogs with physicochemical and sensory properties closely mimicking those of real meat products (such as burgers, sausages, crumbles, and nuggets) has had a profound impact on the modern food industry (Sha & Xiong, 2020). The market value of companies such as *Impossible Foods* and *Beyond Meat* has rapidly grown in recent years (Crosser, 2020), which has attracted attention from entrepreneurs, investors, and other food companies. As a result, a growing number of start-up companies has arisen in the plant-based meat analog space over

the past few years, as well an increasing number of traditional food companies offering more plant-based meat products. The development of high quality products in this area depends on understanding the composition, structure, physicochemical properties, and functionality of real meat, and so we begin by reviewing its properties.

5.2 | Real meat and fish

5.2.1 | Composition and structure

Animals, such as cows, pigs, sheep, chickens, and fish, have been used as a source of food throughout human history and pre-history (Standage, 2009). Most of the organs and tissues found in animals have been used for this purpose, including limbs, breast, back, abdomen, kidneys, liver, heart, brain, tongue, skin, and bones. However, the most commonly consumed meat products in many countries are comprised of muscle fibers, connective-, and adipose tissue, and so these constituents will be the focus of this section.

Animal tissue is organized into complex hierarchical structures within meat (Figure 4), which contribute to the physicochemical properties and multisensory experience when preparing and consuming meat products (Prayson, McMahon, & Prayson, 2008). An understanding of the structural organization and interactions of the various constituents within meat, as well as their impact on the overall physicochemical, functional and sensory properties of meat, is therefore essential for creating authentic meat analogs.

In living animals, the main function of *muscle fibers* is to enable the contraction and expansion of muscles so that the creature can move around (Toldra, 2017). Muscle fibers are comprised of bundles of thin protein-rich filaments that provide both mechanical rigidity and flexibility to the muscles of live animals. They also contribute to the desirable textural attributes and mouthfeel of meat products. These fibers are primarily assembled from two kinds of proteins, *actin* and *myosin*, which are organized in a manner that enables the contraction and expansion of the muscles driven by energy-generating cellular processes. The directional alignment of meat fibers within muscle tissues leads to many of their unique textural attributes, including their disassembly within the mouth during mastication (Lillford, 2016). The structural organization of these fibers is typically altered during the processing of meat, which contributes to the different textural and mouthfeel attributes of processed meat products, such as sausages and burgers (Tornberg, 2013; Tornberg et al., 2000). In addition, it is altered by cooking, leading to changes in the texture and fluid holding properties of meat products (Ertbjerg & Puolanne, 2017). The various proteins in muscle tissue undergo thermal transitions during cooking that alter meat texture, such as denaturation of myosin heads (40 to 50°C), myosin rods and light chains (~54°C), sarcoplasmic proteins (~65°C) and actin (~80°C) (Bertram, Wu, van den Berg, & Andersen, 2006).

Connective tissue helps to hold the various constituents within muscles together (Toldra, 2017). It forms a thin sheet around the muscle fibers that holds them within bundles inside the muscles (Figure 4). The strength and amount of connective tissue present in an animal increases as it ages, which impacts meat quality. The main structural component of connective tissue is *collagen*, which exists as a triple helix comprised of three intertwined polypeptide chains (Figure 3) (Lepetit, 2008). The formation of helices is favored by the relatively high proline and hydroxyproline content of the collagen molecule. At the body temperature of an animal, collagen is a long rigid rod-like molecule that provides mechanical strength and flexibility to connective tissue. Nevertheless, the triple helices unravel when collagen is heated above about 62 to 67°C, and the polypeptide molecules that are released adopt a more flexible random-coil arrangement (Purslow, 2018). This is one of the reasons that meat changes its texture when it is heated during cooking.

In living animals, *adipose tissue* acts as a fat reservoir, which can be utilized as an energy source to power the muscles and other bodily functions (Hausman, Bergen, Etherton, & Smith, 2018). It also plays an important role in maintaining nutrient and energy homeostasis by releasing various hormones (Stern, Rutkowski, & Scherer, 2016). Structurally fat cells (adipocytes) consist of a pool of triacylglycerols trapped contained within a cell membrane, with the overall cell dimensions ranging from tens to hundreds of micrometers (Urrutia et al., 2018). Adipose tissue is found in various locations within an animal, such as below the skin and as distinct regions within the muscle. In meat products, this latter kind of adipose tissue is often referred to as "marbling". The type, level, and location of the adipocytes play an important role in determining their physicochemical and sensory attributes, such as texture, mouthfeel, and flavor (Joachim & Schloss, 2018). A confocal microscopy image of the large fat cells in beef adipose tissue is shown in Figure 9.

Humans consume meat in many different forms and some of them are easier to simulate with plant-based ingredients than others. Whole muscle products, such as a beef steak, pork chop, lamb shank, or chicken breast, have complex structural hierarchies that are fairly close to those found in the flesh of a living animal. In contrast, comminuted products, such as ground beef, sausages, burgers, and nuggets, have structures that are very different from those found in the animal and these are much easier to simulate

using plant-based ingredients (such as TVPs) than whole muscle products. The simulation of whole muscle tissues is an area where there is still an urgent need for research.

5.2.2 | Physicochemical and functional properties

The physicochemical and functional properties of whole muscle tissue depend on the animal it was taken from, such as cow, pig, sheep, chicken, or fish, as well as the part of the animal used, such as shoulder, chest, stomach, back, leg, or rump (Sun & Holley, 2011). Nevertheless, there are many similarities in behavior because they all contain fairly similar structural units and microstructures. Whole meats are optically opaque semi-solid materials, whose color depends on the type and level of natural pigments present (such as myoglobin). The opacity of meat products is due to the scattering of light waves by the muscle fibers and adipose tissue, since they have dimensions that fall within the wavelength of visible light. The semi-solid nature is due to the presence of a structurally complex network of protein molecules that form crosslinks with each other through physical interactions, such as van der Waals forces, hydrogen bonding, electrostatic attraction, and hydrophobic bonds (Acton & Dick, 1984; Xiong, 1994). At present, the compositional and structural complexity of muscle proteins, as well as the diverse range of molecular interactions involved, makes it extremely difficult to develop a full understanding of the molecular basis of their physicochemical and functional properties.

Ideally, it is important to mimic the properties of a meat product before, during, and after cooking. For instance, a beef steak analog should change from shiny, pinky-reddish, and tough before cooking to matt, brownish, tender and juicy after cooking, and this transition should occur under the same time-temperature conditions as seen with a real beef steak. Consequently, it is important to understand the behavior of meat throughout the cooking process.

Cooking loss and textural changes: Cooking loss is a measure of the reduction in the mass of a piece of meat during cooking, as a result of leakage of meat fluids, which typically contain water, proteins, minerals, and lipids. The loss of these fluids is a major reason for the visible shrinking of the meat and a change in its tenderness and juiciness. The texture of meat also changes when it is cooked as a result of changes in the structural organization and interactions of the various kinds of proteins present (Hughes, Oiseth, Purslow, & Warner, 2014; Yu, Morton, Clerens, & Dyer, 2017). Typically, the myosin (40 to 60°C), then the sarcoplasmic proteins (50 to 70°C) and then the actin (70 to 80°C) molecules denature during heating, which causes the muscle fibers to progressively disassemble (Bertram et al., 2006; Zielbauer, Franz, Viezens, & Vilgis, 2016). These changes in the structural organization cause the muscle fibers to shrink in width (50 to 65°C) and then in length (70 to 75°C), causing some of the entrained fluids to be forced out, as well as toughening the meat. Conversely, softening of the meat may occur during heating due to unraveling of the triple helix structure of the collagen molecules in the connective tissue (60 to 70°C). Knowledge of these molecular and structural events and their impact on the texture and fluid holding properties of meat is essential when designing meat analogs with similar cooking properties to real meat.

Appearance: The alteration in the appearance of meat products during cooking is also governed by molecular and structural changes (Toldra, 2017). Prior to cooking, raw whole muscle tissues tend to have a shiny, opaque pinkish appearance. The shininess is a result of light waves being specularly reflected from the smooth wet surfaces. Conversely, the matt appearance of cooked meat is because its surfaces have dried and become rougher. The opaque appearance of meat is a result of light being scattered from the protein fibers and fat cells. Moreover, the pink color of raw meat is a result of selective light absorption by myoglobin, which is a globular protein that contains a heme group. The heme group has an iron atom at its center that has lost either two (Fe^{2+}) or three (Fe^{3+}) electrons depending on the environmental conditions. In the living animal, myoglobin binds oxygen and stores it in the muscle tissues until it is required for energy generation. In meat, the color of myoglobin (red, purple, or brown) depends on the oxidation state of the iron in the heme group, as well as whether the heme binds oxygen or not. At ambient temperature, the heme group contains Fe^{2+} and strongly binds oxygen, leading to a reddish color. However, when myoglobin is heated above about 60°C it undergoes a structural transition that leads to the release of the oxygen and the conversion of Fe^{2+} to Fe^{3+}, thereby causing the color to change to brown. Finally, the dark brown crust on well-cooked meat is largely the result of the Maillard reaction, which involves a complex series of transitions between proteins and sugars at the high temperatures and low moisture contents at the surfaces of meat during cooking.

Flavor profile: The unique flavors of meat products are a combination of aroma, taste, and mouthfeel attributes. The aroma and taste of meat are due to volatile and nonvolatile molecules that react with the flavor sensors in the human nasal and oral cavities. Many of these molecules are generated within the meat during the cooking process, especially by Maillard and lipid oxidation reaction products (Kosowska, Majcher, & Fortuna, 2017). The flavor profile of a meat product depends on the composition of the raw meat (e.g., amino acids, fatty acids,

monosaccharides, minerals), as well as the cooking processes used (e.g., frying, steaming, broiling, grilling, and temperature-time profile). The mouthfeel of meat is due to physical sensations that arise when it interacts with the surfaces of the tongue and palette during mastication, and results in sensory attributes like tenderness, juiciness, granularity, and smoothness (Dreeling, Allen, & Butler, 2000; Roth, McKeith, & Brewer, 1999). During chewing the complex internal structure is progressively dissembled, which contributes to its desirable mouthfeel (Lillford, 2016).

5.2.3 | Plant-based meat and fish analogs

In this section, some of the key factors that need to be accounted for when developing plant-based meat analogs that simulate the properties of real meat are given. A more detailed review of the science behind meat analogs has been provided in a number of recent review articles that focus specifically on this topic (Ismail, Hwang, & Joo, 2020; Kyriakopoulou et al., 2019; Malav, Talukder, Gokulakrishnan, & Chand, 2015).

Plant-based meat analogs are mainly constructed from plant-based proteins, polysaccharides, and lipids but they also contain other functional ingredients, such as colorants, flavorings, minerals, vitamins, and preservatives (Sha & Xiong, 2020). The type and concentration of these ingredients, as well as the processing methods used to assemble them, determine the physicochemical and sensory attributes of the final product and need to be optimized for each kind of meat product that is being simulated.

Texture: As mentioned earlier, the textural properties of comminuted meat products, such as sausages, burgers, or nuggets, can often be simulated fairly accurately using texturized vegetable proteins (TVPs) (Ismail et al., 2020). It is much harder to mimic the unique textural attributes of whole muscle foods, such as beef steaks, chicken fillets, and pork chops, using plant-derived ingredients. The meat analogs should simulate the texture of whole meats throughout cooking, as well as breaking down within the oral cavity during mastication to give a similar mouthfeel to real meat.

Researchers are examining a variety of approaches of assembling plant-derived proteins and other ingredients into structures that mimic those found in real meat, thereby simulating many of their textural attributes. As discussed earlier, these can be divided into physicochemical or processing operations, which can be used separately or in combination (Zahari et al., 2020). Physicochemical operations typically involve controlled phase separation, structuring, and gelation of biopolymer mixtures, usually proteins and polysaccharides (Kyriakopoulou et al., 2019). Using appropriate compositions (biopolymer types and concentrations) and solution conditions (pH, ionic strength, and temperature) a biopolymer mixture spontaneously separates into two phases (Figures 10 and 11). This phase-separated mixture can then be sheared to create fiber-like structures that are then gelled by changing environmental conditions (such as heating or cooling) or by adding crosslinking agents (such as minerals or enzymes) (Figure 12). As a result, meat-like structures can be formed that have some of the desirable physicochemical attributes of real meat (Figure 9). In contrast, processing approaches involve creating structures by using specific types of processing operations, such as extrusion or shearing (see earlier). For instance, high moisture extrusion has been used to create meat-like textures and structures from a variety of different plant ingredients including peanut, hemp, soy, microalgae, pea, wheat gluten, faba bean, and lupin proteins, which has been reviewed recently (Cornet et al., 2021b). Similarly, shearing methods have been used to produce chicken-like textures from blends of soy or pea proteins with gluten (Schreuders et al., 2019a). Additionally, the adipose tissue in pork fat has been simulated by forming emulsion gels from olive oil and chia mucilage (Camara et al., 2020).

Fluid holding: The ability of plant-based meat analogs to retain fluids before, during, and after cooking influences their appearance, cookability, texture, and mouthfeel. The fluid holding properties of meat analogs depend on biopolymer-solvent interactions, the elastic strength of the biopolymer gel network, and the osmotic pressure associated with an uneven distribution of ions inside and outside the gel network (Cornet et al., 2021a). In a recent study, the impact of pH and ionic strength of a marinade on the water holding capacity (WHC) of meat analogs produced by a shear cell was determined (Cornet et al., 2021a). The WHC of the meat analogs increased as the ionic strength decreased and as the pH increased above the protein's isoelectric point, which was attributed to a strengthening of the electrostatic interactions in the system. The WHC of meat analogs may be altered by changing the nature of the biopolymers used, such as their molecular weights, polarities, or cross-linking densities. For instance, it was shown that the swelling and WHC of meat analogs formed from plant proteins decreased as their degree of crosslinking was increased by adding chemical agents, such as glutaraldehyde (Cornet et al., 2021a). Alternatively, the addition of plant-based dietary fibers, such as carrageenan, pectin, or alginate may improve the fluid holding properties of meat analogs, as in real meat products (Talukder, 2015).

Flavor: The creation of meat analogs that have flavor profiles similar to those of specific meat products requires

identification of the key aromatic and taste molecules. Meat contains hundreds of different volatile molecules, but only some of these contribute to their characteristic meaty flavor (Kosowska et al., 2017). The aroma of cooked meat depends on "meaty" flavor notes such as 2-methyl-3-furantiol or bis-(2-methyl-3-furan) disulfide, as well as other flavor compounds that have "green," "mushroomy," "sweet," and "earthy" odors. The taste of cooked meat perceived by the tongue mainly depends on the balance of umami, salty, sweet, and sour components, which may be present in the animal flesh originally or generated during cooking.

Once the key flavor constituents in a real meat product have been identified, then it is important to mimic them within a plant-based meat analog. Meaty flavor profiles can be created in meat analogs using a variety of approaches. Leghemoglobin isolated from soybean roots or microbial fermentation contributes to the desirable "meaty" flavor of some commercial plant-based meat products, notably those produced by Impossible Foods. Presumably, the heme iron becomes more exposed when the protein is denatured during heating, which promotes oxidation reactions, thereby generating aroma compounds similar to those produced in real meat during cooking (Fraser, Shitut, Agrawal, Mendes, & Klapholz, 2018). Mycoprotein is also being explored because it has been reported to give a meaty aroma, a savory umami taste, and a meat-like texture (McHugh & Avena-Bustillos, 2019). In seafood applications, seaweed, algae, and microalgae are often used as ingredients because they have seafood-like flavor attributes (McHugh & Avena-Bustillos, 2019). Furthermore, ingredients with meaty flavors that can be used in plant-based meat analogs can also be produced by carrying out controlled Maillard and oxidation reactions on plant-based ingredients (Watson, 2019). In some cases, substrates containing iron are employed in these reactions to obtain volatile reaction products that simulate some of the characteristic aromas produced in meat due to the presence of heme iron. An important consideration when developing these flavors is that they are not lost due to evaporation or chemical degradation under the hot conditions generated in food processing operations such as extrusion or shearing (Watson, 2019). It is also important to take into account that the flavors added may bind to plant proteins or become solubilized within fat droplets during processing, thereby reducing their intensity. In contrast, many plant proteins have unpleasant flavors, such as the astringent, earthy, or vegetative notes of mung bean, peas, and chickpeas (Watson, 2019). For this reason, researchers are working to reduce these off flavors using plant-breeding, processing, or formulation methods.

Appearance: Ideally, a meat analog should mimic the appearance of the meat product it is designed to simulate before, during, and after cooking. The opacity of meat analogs requires that they contain fibers and/or particles with dimensions that lead to strong light scattering (200 to 2000 nm). The surface sheen of meat analogs depends on their surface roughness, which depends on the structural organization of the different ingredients at their surfaces. Ideally, a plant-based milk analog should go from shiny before cooking to matt after cooking, which may occur because the dehydration of the surface can make it become rougher, thereby scattering light waves more diffusely. The color of meat analogs can be controlled by including pigments that preferentially absorb light waves in an appropriate wavelength range. Typically, they should give a pinkish/red color for the raw product and a brownish color for the cooked product. However, in many cases it may only be necessary to mimic the desirable color of the final cooked product, for example, frozen microwavable foods.

In commercial plant-based meat analogs, a number of approaches have been used to obtain a color that mimics real meat. For instance, beet juice extract, which contains the water-soluble pigment betalain, has been used for this purpose in Beyond Meat's products. Studies of its thermal stability have shown that beet juice changes color from red-violet to orange-yellow when heated, which was attributed to chemical degradation of the betalain (Herbach, Stintzing, & Carle, 2004; Kayin, Atalay, Akcay, & Erge, 2019). A variety of other natural pigments, such as the aforementioned leghemoglobin, with different color characteristics are available for use in plant-based meat analogs, which can be used individually or in combination to obtain the desired product appearance (Juric et al., 2021).

5.3 | Nutritional, sustainability, and ethical comparison

As mentioned earlier, many consumers adopt a plant-based diet for health, sustainability, and/or ethical reasons. Some people believe that plant-based foods are healthier for them. For this reason, we compared the nutritional profiles of two meat analogs (Impossible and Beyond Meat burgers), a black bean burger (Greenwise), and a real beef burger reported in the USDA food data base (Table 5). It should be stressed that simply comparing the gross macronutrient compositions of meat and meat analogs does not provide an adequate consideration of differences in their overall nutritional impacts. For instance, there are differences in the types and concentrations of essential amino acids, fatty acids, vitamins, and minerals present, as well as differences in the rate and extent of macronutrient digestion and absorption, which impact their metabolic and physiological responses.

TABLE 5 Comparison of nutrient content of meat burgers, plant-based meat burger analogs, and black bean burgers

	Percentage content (w/w)			
	Beef burger	**Plant-based (Impossible)**	**Plant-based (Beyond)**	**Bean burger (Greenwise)**
Calories (kcal)	212	212	257	197
Total fat	15.0	12.4	19.5	9.9
Saturated fat	5.3	8.0	6.2	0.7
Trans fat	0.9	0	0	0
Unsaturated fat	9.8*	4.4*	13.3*	9.2*
Cholesterol	0.066	0	0	0
Sodium	0.066	0.33	0.30	0.45
Total carbohydrate	0	8.0	3.5	21.1
Dietary fiber	0	2.7	0.9	4.2
Sugars	0	0.9	0	4.2
Protein	18.6	16.8	15.9	8.5

Note: Values are given per 100 g of product. Information obtained from the USDA FoodData Central database (https://fdc.nal.usda.gov/). There will be appreciable differences in these values depending on the origin of the animal and plant-based products compared.

Impossible Foods—Ingredients: Water, soy protein concentrate, coconut oil, sunflower oil, natural flavors; 2% or less of: potato protein, methylcellulose, yeast extract, cultured dextrose, food starch modified, soy leghemoglobin, salt, soy protein isolate, mixed tocopherols (vitamin E), zinc gluconate, thiamin hydrochloride (vitamin B1), sodium ascorbate (vitamin C), niacin, pyridoxine hydrochloride (vitamin B_6), riboflavin (vitamin B_2), vitamin B_{12}.

Beyond Meat—Ingredients: Water, pea protein, expeller-pressed canola oil, refined coconut oil, rice protein natural flavors, methylcellulose, potato starch, apple extract, pomegranate extract, salt, potassium chloride, vinegar, lemon juice concentrate, sunflower lecithin, beet juice extract (for color). Carrot.

Greenwise black bean burger—Ingredients: Cooked black beans (black beans, water), cooked brown rice (brown rice, water), onions, corn, potato flakes, expeller pressed canola oil, red peppers, wheat gluten, jalapeno peppers, garlic, cilantro, salt, chili powder (chili pepper, salt, garlic, cumin, spices), chipotle pepper powder, black pepper.

*The unsaturated fat was calculated as total fat minus saturated and trans fat.

TABLE 6 Comparison of the environmental impact of beef patties and plant-based beef patty analogs

Impact category	Units	Beyond burger	Beef patty	% Reduction
Greenhouse gas	kg CO_2 eq.	0.4	3.7	89.2
Energy Use	MJ	6.1	11.4	46.5
Land Use	m^2 a eq.	0.3	3.8	92.1
Water Use	L eq.	1.1	218.4	99.5

Based on Heller and Keoleian. (2018).

There are pronounced differences in the nutrient compositions of the different products, which could have some health implications when they are consumed as part of a long-term diet. For instance, the Beyond Meat burger has a higher calorie, total fat, saturated fat, and salt content than the real beef burger, as well as a lower protein content. The Impossible burger has a considerably higher saturated fat, carbohydrate, and salt content than the real beef burger, as well as a lower protein content. The black bean burger has a much lower total and saturated fat content than the real beef burger, but a much higher carbohydrate and salt content. The relatively high saturated fat content of the Impossible burger can be attributed to the use of coconut oil as a fat source. The higher carbohydrate content of the meat analogs are due to the use of sugars and starches as additives. Interestingly, the Impossible burger contains a variety of vitamins and minerals to enhance its nutritional profile, whereas the Beyond Meat burger and Greenwise black bean burger do not. There are also appreciable differences between the amino acid compositions of the proteins in the different burgers. As discussed earlier, meat proteins contain a better balance of essential amino acids than plant-based ones (Table 3) (Herreman et al., 2020). The Impossible burger contains a blend of soy and potato protein, which would help to improve the overall nutritional quality of the product by providing all the essential amino acids required.

These nutritional comparisons show that there are appreciable differences between plant-based products, and that just because a product is constructed from plant-derived ingredients does not necessarily make it healthier. It is clear that further work is required to improve the nutritional profiles of plant-based meat analogs, while still making them affordable, sustainable, and delicious. Moreover, it will be important to establish differences in their digestibility and bioavailability characteristics, as these factors also impact human health and wellbeing.

Another major driving force for consumers adopting a more plant-based diet is the potential improvement in sustainability. A life cycle analysis (LCA) has been carried out that compared the environmental impact of the Beyond Meat burger with a real beef burger (Heller & Keoleian, 2018). The results of this study are summarized in Table 6, which shows that the plant-based burger uses 47% less energy, produces 89% fewer greenhouse gasses, 92% less land, and 99% less water. Similarly, a LCA of the environmental impact of an Impossible burger and beef burger reported that there was 92% less eutrophication, 89% fewer greenhouse gasses, 96% less land use and 87% less water use for the plant-based burger compared to the animal-based one (https://impossiblefoods.com/sustainable-food/burger-life-cycle-assessment-2019). These comparisons demonstrate that including more plant-based meat analogs within the human diet could have a major benefit on the sustainability of the modern food supply.

Finally, many consumers do not want to consume meat because of ethical reasons associated with the confinement and slaughter of animals. The United Nations Food and Agriculture Organization (FAO) estimates that there were around 302 million cows slaughtered for food in 2018 (www.fao.org/faostat/). Although there has been considerable progress in the more humane treatment of livestock animals in many regions of the world over the past few decades, there are still problems associated with their confinement (Grandin, 2018) An increase in the number of plant-based meat analogs consumed would reduce the number of animals used as livestock.

5.4 | Eggs

Plant-based egg analogs are another successful product that has recently been introduced into the market. For instance, *Just*, a food company based in San Francisco (California), creates fluid eggs (JUST Egg™) and egg slices (JUST Egg Folded™) that are designed to mimic the properties of real egg products (Table 7). The creation of these egg analogs requires a good understanding of the composition, structure, and properties of real egg. For this reason, we start this section by reviewing the characteristics of real hen's eggs and then discuss the science behind creating egg analogs.

TABLE 7 Comparison of nutrient content of hen's eggs and plant-based hen's egg analogs

	Percentage content (w/w)		
	Plant-based egg	**Plant-based egg folded**	**Hen's egg**
Calories per serving (kcal)	77 kcal/44 mL	100 kcal/57 g	72 kcal/50 g
Calorie density (kcal per 100 g)	175	175	144
Total fat	11.4	12.3	10
Saturated fat	0.0	0.9	3.2
Trans fat	0.0	0.0	0
Polyunsaturated fat	3.4	3.5	1.8
Monounsaturated fat	6.8	7.9	3.6
Cholesterol	0.0	0.0	0.411
Sodium	0.386	0.526	0.129
Total carbohydrate	2.3	5.3	1
Dietary fiber	0.0	0.0	0
Sugars	0.0	0.0	0.2
Protein	11.4	12.3	12

Information obtained from the USDA FoodData Central database (https://fdc.nal.usda.gov/).
Note: Values are given per 100 g of product. The plant-based egg products are both manufactured by Just Foods (San Francisco, CA). There will be appreciable differences in these values depending on the origin of the animal and plant-based products compared.
Ingredients: *JUST Egg (fluid)*: Water, mung bean protein isolate, expeller-pressed canola oil, contains less than 2% of dehydrated onion, gellan gum, natural carrot extractives (color), natural flavors, natural turmeric extractives (color), potassium citrate, salt, soy lecithin, sugar, tapioca syrup, tetrasodium pyrophosphate, transglutaminase, nisin (preservative) (contains soy). *JUST Egg Folded (semi-solid)*: Water, mung bean protein isolate, expeller-pressed canola oil, corn starch, contains less than 2% of baking powder (sodium acid pyrophosphate, sodium bicarbonate, corn starch, monocalcium phosphate), dehydrated garlic, dehydrated onion, natural carrot extractives (color), natural turmeric extractives (color), salt, transglutaminase.
Hen's egg: Grade A, large whole egg.

5.5 | Real hen's eggs

5.5.1 | Composition and structure

The main constituents of hen's eggs are water (75%), proteins (12%), and lipids (12%), with lower levels of carbohydrates and minerals (Kovacs-Nolan, Phillips, & Mine, 2005). Structurally, hen's eggs are comprised of an opaque yellow semi-solid core known as the yolk (≈ 27.5%) surrounded by a transparent viscous fluid known as egg white (≈ 63%), which is contained within a hard calcium carbonate-rich shell (≈ 9.5%). The proteins are distributed throughout the egg, with the majority being found in the yolk and white, and only a small fraction being located within the eggshell (Kovacs-Nolan et al., 2005). Both egg white and yolk contain numerous kinds of proteins, which may be physically or covalently attached to carbohydrates or lipids in their natural environment (Table 1b). Egg yolk is a complex colloidal dispersion consisting of lipoprotein-rich granules suspended within a protein-rich aqueous phase that also contains water-soluble vitamins and minerals. Egg yolk can be separated into granules and plasma by centrifugation, with each fraction containing different components and functional properties (Huang & Ahn, 2019). The main macronutrients in egg yolk are lipids (27%), proteins (16%), and carbohydrates (4%), which may be free or assembled into complex multicomponent structures (Brady, 2013). Indeed, the majority of proteins in egg yolk are contained in low density lipoproteins (68%), high density lipoproteins (12%), livetins (12%), and phosvitin (7%). Thus, many of the egg yolk proteins are integral parts of colloidal particles (lipoproteins), which consist of a triglyceride and cholesterol-rich core surrounded by a layer of phospholipids and proteins. Egg yolk is also a rich source of vitamins (A, B_1, B_2, B_5, B_9, D, and choline) and minerals (phosphorous, zinc, iron, and calcium). In contrast, egg white is a hydrocolloid solution consisting of proteins and glycoproteins dispersed within an aqueous medium that contains water-soluble vitamins (riboflavin) and minerals (selenium) (Brady, 2013). Egg white typically consists of around 90% water and 10% protein, with only minor amounts of lipids, sugars, vitamins, and minerals.

5.5.2 | Physicochemical and functional properties

Eggs may be used as functional ingredients in a variety of different forms, including whole eggs, egg whites, egg yolks, and blends, which may be supplied as fluids or powders (McGee, 2004). In this section, we focus on the properties of fluid whole eggs, which consist of egg whites and yolks blended together, since this is currently the most

common type of product that plant-based egg analogs are designed to simulate (Table 7). Under ambient conditions, these products are viscous fluids with a uniform cloudy yellow appearance. The viscous nature of these products is a result of the presence of relatively high concentrations of proteins and glycoproteins, which act as thickening agents. The yellowish color comes from the presence of carotenoids, such as β-carotene, lutein and zeaxanthin, which are natural hydrophobic pigments that arise from the chicken feed. The optically opaque appearance of fluid whole egg is a result of light scattering by colloidal particles, such as lipoprotein-rich granules from the egg yolk, which have dimensions similar to the wavelength of light.

Eggs are widely used in cooking because of their desirable taste, texture, and functional properties (Vega & Mercade-Prieto, 2011). In particular, they contain globular proteins that are able to thicken solutions or form gels when they are heated above their thermal denaturation temperatures (Table 1b), as well as adsorb to oil-water and air-water interfaces and stabilize emulsions and foams. Consequently, any plant-based ingredients used in egg analogs should be able to perform some or all of these functions, depending on the application. One of the most difficult attributes of real eggs to simulate is their versatility. They can be used in a wide range of food applications, including alone (boiled, poached, fried, and scrambled), or as part of baked goods, creams, desserts, dressings, mayonnaise, meringues, and sauces (McGee, 2004).

The proteins in egg white and yolk tend to unfold when they are heated above their thermal denaturation temperatures, which range from around 63 to 93°C (Table 1b). After the proteins unfold, they expose non-polar and sulfhydryl groups that can form crosslinks through hydrophobic attraction and disulfide bonding, which leads to the creation of a semi-solid 3D protein network (Kiosseoglou & Paraskevopoulou, 2005). Different textures ("soft" to "hard") can be created by heating eggs at different temperatures and times (Vega & Mercade-Prieto, 2011).

The thermal denaturation temperatures of many plant proteins are above the most common proteins found in eggs (Tables 1b and 1d), which means they typically have to be heated to a higher temperature and for longer before they can form gels. Egg white and yolk form gels with different characteristics because of their different compositions and structures. The gels formed from egg white tend to be glossy white and rubbery, whereas those formed by egg yolk tend to be matt yellow and crumbly. The gels formed by blends of egg white and egg yolk have characteristics somewhere between those formed by the two individual components. Consequently, plant-based alternatives must be designed to mimic the properties of the specific egg product they are intended to replace.

Raw eggs have little aroma, but they may gain a mild sulphury smell when they are cooked due to thermal degradation and chemical reactions of sulfur-containing amino acids (Jo et al., 2013; Warren, Larick, & Ball, 1995). Gas chromatography-mass spectrometry (GC-MS) methods have been used to identify the key volatile components in raw eggs (Xiang, Jin, Gouda, Jin, & Ma, 2019) and cooked eggs (Goldberg et al., 2012; Jo et al., 2013). This knowledge is useful for identifying plant-based ingredients that may provide similar flavor profiles as real eggs.

5.5.3 | Plant-based egg analogs

Plant-based egg analogs are usually assembled from globular plant proteins capable of undergoing a sol-gel transition when they are heated. Ideally, the thermal transition temperature of the plant proteins should be fairly similar to that of real egg proteins (i.e., around 63 to 93°C), which is often challenging because many plant proteins have higher denaturation temperatures (Table 1d). As a result, egg alternatives containing plant proteins often have to be heated to a higher temperature and/or for longer to form a gel that resembles that formed by real egg. It is therefore important to identify a combination of plant proteins that unfolds and aggregates over a similar temperature range as egg proteins, that produces a similar texture-temperature profile, and that produces a similar final appearance and texture. Consumers can then use the same cooking conditions for egg analogs as they would use for real eggs.

Many plant proteins are able to form gels when they are heated above their thermal denaturation temperatures, such as soybean, pea, chickpea, bean, and sunflower, but the protein structure, protein concentration, salt, pH, and temperature conditions must be carefully controlled (Hettiarachchy, Kannan, Schäfer, & Wagner, 2013). The globular proteins in egg analogs may also provide emulsifying and foaming properties in products where these attributes are needed, such as dressings, sauces, or meringues.

Egg analogs may also require various other ingredients to simulate specific attributes of real eggs. An emulsified plant-based oil may be included to provide desirable optical, textural, mouthfeel, and flavor properties. These oil droplets simulate some of the functional attributes normally provided by the lipoproteins in real eggs. Thickening or gelling agents may be added to modify the texture and inhibit the sedimentation or creaming of particulate matter, especially when the egg analog is in a fluid state. Natural pigments, such as curcumin or carotenoids, are typically added to mimic the desirable yellowish color of egg. A variety of flavorings may also be added to obtain an egg-like aroma and taste, such as sugars, salts, herbs, or spices.

Commercial egg analogs have been developed that mimic both fluid and semi-solid whole egg products, such as the JUST Egg™ and JUST Egg Folded™ products mentioned earlier (www.ju.st). The fluid eggs come in a plastic container and can be used to create scrambled eggs, omelets, or French toast in a frying pan, whereas the folded egg slices come in frozen squares that can be heated in an oven, microwave, skillet, or toaster and then used in breakfast sandwiches. In both cases, the main ingredient responsible for the semi-solid texture of the cooked "egg" products is mung bean protein, which forms a gel when heated due to thermal denaturation and aggregation of the globular mung bean proteins. The yellowish color of these products comes from natural pigments, such as curcumin from turmeric and carotenoids from carrots. The opacity and textural characteristics are also influenced by the presence of emulsified canola oil. Various other ingredients are also present in these products as texture modifiers/stabilizers (such as corn starch and gellan gum), flavorings (such as garlic, onion, sugar, and salt), pH modulators (such as bicarbonates, citrates, or phosphates) and preservatives (such as nisin). These products also contain transglutaminase, which is an enzyme that crosslinks proteins, thereby increasing the gel strength of the end product (Gharibzahedi et al., 2018).

5.5.4 | Nutritional, sustainability, and ethical comparison

The nutritional profiles of commercially available egg analogs and real eggs are compared in Table 7. The egg analogs have a higher calorie, total fat, polyunsaturated fat, salt, and carbohydrate content than the real eggs but a lower saturated fat content. The total protein contents of all the products were fairly similar. However, as mentioned earlier, the nutritional quality of egg proteins is higher than that of plant proteins, such as the mung bean proteins used in the egg analogs, because they contain the full complement of essential amino acids required (Table 3). As mentioned earlier, in the meat analog section, egg analogs may also have different metabolic and physiological responses to hen's eggs over and above differences in their macronutrient contents, which have to be accounted for. Differences in the nutritional profiles of egg analogs and real eggs could have implications for human health but the magnitude of these effects is currently unknown.

A life cycle analysis carried out for Just Foods reported that there was 99% less water use, 86% less land use, and 94% less greenhouse gas emissions for the egg analogs than for real eggs (https://www.ju.st/stories/impact). However, additional independent life cycle analyses should be carried for this and other plant-based egg analogs to assess their environmental impact. Additionally, it has been estimated that nearly 69 billion chickens were slaughtered globally in 2018, which represents a combination of birds used for meat and for eggs (https://ourworldindata.org/meat-production). There may therefore be substantial sustainability and ethical benefits associated with including more plant-based eggs within the human diet.

5.6 | Milks and creams

In terms of market size, milks are the most popular plant-based foods, currently making up over 40% of the total market in the United States (Figure 1) (Crosser, 2020). Structurally, plant-based milk analogs are complex colloidal dispersions that consist of small particles and biopolymers dispersed within an aqueous solution (McClements et al., 2019). These particles may be oil bodies, fat droplets, plant tissue fragments, and/or insoluble calcium carbonate particles. Plant-based milk analogs are often designed to mimic the physicochemical, functional, and sensory attributes of cow's milk. In this section, we begin by describing the properties of real cow's milk and then discuss plant-based alternatives. More detailed information on the science and technology of plant-based milk analogs are provided in a number of recent review articles (Aydar, Tutuncu, & Ozcelik 2020; McClements, 2020a; McClements et al., 2019; Sethi, Tyagi, & Anurag, 2016).

5.7 | Real cow's milk

5.7.1 | Composition and structure

Raw cow's milk is a natural colloidal suspension comprising of small milk fat globules and casein micelles dispersed within an aqueous phase containing lactose, oligosaccharides, whey proteins, and salts (Jukkola & Rojas, 2017). Raw milk is typically processed before sale to improve its shelf-life and safety using processing operations such as homogenization, separation, pasteurization, and/or sterilization (Campbell & Marshall, 2016). Compositionally, raw milk typically consists of around 87% water, 4% to 5% lactose, 3% to 4% fat, 3% protein, 0.8% minerals, and 0.1% vitamins (Pereira, 2014). The milk fat globules in raw milk have a hydrophobic triglyceride-rich core surrounded by an amphiphilic interfacial layer containing phospholipids, proteins, cholesterol, and other surface-active molecules (Lopez, Cauty, & Guyomarc'h, 2015; Verma et al., 2019). These milk fat globules have mean diameters of around 5 μm and a moderate negative charge (−10 mV), which is mainly due to the presence of anionic phospholipids and proteins at their surfaces. Oil soluble vitamins and

carotenoids may also be located within the hydrophobic cores of the milk fat globules. After homogenization, the size of the oil droplets is decreased appreciably (<500 nm), which greatly improves their stability to creaming during storage (Lopez et al., 2015). Moreover, the interfacial composition is changed, so that the milk fat droplets are primarily surrounded by a layer of proteins, mainly casein molecules and micelles (Obeid et al., 2019). As a result, the casein-coated fat globules in homogenized milk have a surface potential (−16.5 mV) that is fairly similar to that of casein (−15.6 mV).

The casein micelles in bovine milk are colloidal particles (50 to 500 nm) assembled from α_{S1}, α_{S2}, β, and κ caseins, as well as calcium phosphate nanoclusters, with hydrophobic and electrostatic attractive forces holding them together (Lucey & Horne, 2018). The surface potential of casein micelles moves from negative to positive when the pH is reduced from neutral to acidic, with a point of zero charge around the isoelectric point of the proteins (pH 4.6) (Obeid et al., 2019). Casein micelles tend to aggregate near their isoelectric point because of the reduction in electrostatic repulsion between them. In addition, they may aggregate if the glycopeptide segment of κ-casein is enzymatically cleaved by rennet during cheese manufacture, as this reduces the steric repulsion between neighboring casein micelles. The presence of milk fat globules and casein micelles plays a critical role in determining the physicochemical and functional properties of cow's milk. A range of dairy products with different fat contents are produced by centrifuging cow's milk, including skim, low-fat, reduced-fat, whole milk, light cream, and cream products.

5.7.2 | Physicochemical and functional properties

Cow's milk is an optically opaque white fluid with a relatively low viscosity, and a fairly bland but distinctive flavor profile (Schiano, Harwood, & Drake, 2017). The creamy appearance, mouthfeel, and flavor of milk products are determined by their composition, usually increasing with increasing fat content (McCarthy, Lopetcharat, & Drake, 2017). The flavor profile of milk depends on the diet of the cows, as well as chemical, enzymatic, or microbiological changes occurring during processing and storage (Schiano et al., 2017). The colloidal nature of cow's milk makes it prone to breakdown due to particle flocculation, coalescence, and gravitational separation (Dickinson, 1992). Aggregation can be inhibited by ensuring that there are strong repulsive forces acting between the milk fat droplets and the casein micelles. As mentioned before, under neutral conditions both the fat droplets and micelles have moderate negative charges, which inhibits their aggregation through electrostatic repulsion. But aggregation occurs when the pH is adjusted close to the isoelectric point of the proteins (pH 4.6), since the droplets and micelles lose their charge (Obeid et al., 2019). Controlled aggregation of the casein micelles is a critical step in the formation of dairy products such as yogurt and cheese. Creaming is normally inhibited by ensuring that homogenization leads to sufficiently small milk fat globules (<500 nm) (Lopez et al., 2015a).

Cow's milk is a nutritionally rich food product that contains a diverse range of fats, proteins, carbohydrates, vitamins, and minerals (Chalupa-Krebzdak, Long, & Bohrer, 2018), which may promote human health and wellbeing (Thorning et al., 2016). The potential impacts of switching from cow's milk to milk analogs on human nutrition should therefore be carefully considered. In particular, there is a strong argument for fortifying plant-based milk analogs with micronutrients and nutrients that may be missing from a vegan or vegetarian diet, such as vitamin D, vitamin B_{12}, calcium, and zinc (McClements, 2020a).

5.7.3 | Plant-based milk and cream analogs

The formation, physicochemical, sensory, and nutritional properties of plant-based milk analogs have recently been discussed in detail in a number of review articles (McClements, 2020a; McClements et al., 2019; Sethi et al., 2016). For this reason, only a brief summary of the most important aspects is given here.

Formation, composition, and structure. Typically, plant-based milk analogs are designed to have physicochemical and sensory attributes somewhat similar to that of cow's milk, which means they often have somewhat similar compositions and structures (McClements et al., 2019). Most milk analogs are colloidal dispersions consisting of oil bodies, fat droplets, protein aggregates, and/or plant tissue fragments suspended in an aqueous solution containing dissolved sugars and salts (Figures 13 and 14). These colloidal dispersions may be produced by two different approaches (McClements, 2020a).

First, they may be produced by mechanical or enzymatic disruption of whole plant materials, such as soybeans, coconut flesh, almonds, hazelnuts, oats, or rice (Figure 13). Second, they may be created by homogenization of plant-based oils (such as soy, sunflower, flaxseed, canola, corn, or olive oil) with water in the presence of a plant-based emulsifier (such as proteins, polysaccharides, phospholipids, or surfactants) (Figure 14) (McClements et al., 2019). In both cases the final composition and structure must be carefully controlled to create a final product with the appropriate physicochemical, sensory, and

shelf-life characteristics. In particular, it is important to ensure that the colloidal particles present are not too large, or they will cream/sediment, cause an undesirable gritty mouthfeel, or lead to an inhomogeneous appearance. This can be achieved by selecting appropriate size-reduction conditions during processing, as well as by adding effective emulsifiers and other stabilizers, such as thickening agents. A variety of plant-based ingredients are available for this purpose. Plant-derived proteins (e.g., soy, pea, fava bean, or lentil proteins), polysaccharides (e.g., modified starch), phospholipids (e.g., soy or sunflower lecithin), or surfactants (e.g., quillaja or tea saponins) can be used as emulsifiers (McClements et al., 2017). Plant-derived polysaccharides can be used as thickening agents or stabilizers, such as pectin, locust bean gum, gellan gum, starch, methylcellulose, carrageenan, and alginate (Phillips & Williams, 2021).

Physicochemical and sensory properties: The physicochemical and sensory attributes of plant-based milks are determined by their composition and structural organization (McClements et al., 2019). The creamy appearance of milk analogs is due to the scattering of light waves by the colloidal particles they contain, such as oil bodies, fat droplets, protein aggregates, or plant-tissue fragments. Typically, the lightness of a plant-based milk analog increases as the concentration and refractive index contrast of these colloidal particles increases. Moreover, there is usually a maximum lightness when the particles have diameters that are at least similar to the wavelength of light (~300 to 800 nm). It is therefore important to control particle size and concentration to provide a creaminess similar to that seen in cow's milk. Some plant-based milk analogs contain natural or added pigments that selectively absorb visible light, which leads to a characteristic color, such as yellowish, brownish, or pinkish (Jeske et al., 2019). As a result, they do not have the same appearance as cow's milk, which some consumers find undesirable. For instance, the tristimulus color coordinates of a number of commercial soy milks have been reported to be: $L^* = 62$ to 81, $a^* = -3$ to +3, and $b^* = +7$ to +23 (Liu & Chang, 2013). These values are somewhat different from those reported for cow's milk (skim to full fat): $L^* = 82$ to 86, $a^* = -2$ to −5, and $b^* = +4$ to +8 (McClements et al., 2019). This difference may be desirable or undesirable to consumers depending on their expectations.

Milk analogs are usually designed to be relatively low viscosity fluids like cow's milk, but in some cases, this is not possible. If the colloidal particles within a milk analog are relatively small (<500 nm) so that gravitational separation is not a major issue, then it is possible to have a low viscosity system. Conversely, if the colloidal particles are relatively large, then it may be necessary to add a thickening agent to increase the viscosity so as to inhibit gravitational separation and avoid the appearance of an unsightly cream or sediment layer in the product. To a first approximation, the shear viscosity of a milk analog (η) is described by Einstein's equation:

$$\eta = \eta_1 (1 + 2.5\phi) \tag{9}$$

Here, ϕ is the particle volume fraction and η_1 is the shear viscosity of the aqueous solution surrounding the particles. This equation shows that adding a thickening agent to increase the viscosity of the aqueous phase, will also increase the viscosity of the overall system. As a result, the rheological characteristics of milk analogs can be quite different from those of cow's milk. For example, the shear viscosities of a number of different commercial milk analogs were reported to vary quite considerably (2.2 to 48 mPa s), whereas those of different cow's milks (skim to whole milk) were reported to be fairly similar (2.2. to 2.6 mPa s) (McClements et al., 2019). Presumably, the milk analogs with high viscosities contain thickening agents to inhibit gravitational separation of large particles or to provide a creamy mouthfeel because they do not contain many oil bodies or fat droplets. Cow's milk tends to exhibit Newtonian (ideal) flow characteristics because of the low fat globule concentration, that is, the shear viscosity is independent of applied shear stress (Morison, Phelan, & Bloore, 2013). In the absence of thickening agents, milk analogs also exhibit this kind of behavior. In contrast, in the presence of thickening agents, they exhibit distinct shear-thinning behavior, which will impact their perceived mouthfeel. As an example, the shear viscosity of oat milk has been reported to decrease from around 1400 to 74 mPa s when the shear rate was raised from 1 to 100 s^{-1} (Deswal, Deora, & Mishra, 2014). Strong shear thinning may at least partially account for the perceived "wateriness" of some milk analogs observed after a prolonged time in the mouth reported in sensory tests (discussed later).

Like cow's milk, plant-based milk analogs are colloidal dispersions that may breakdown through a variety of instability mechanisms (McClements et al., 2019). The most important instability mechanisms are particle aggregation and gravitational separation. Aggregation occurs when the attractive forces acting between the colloidal particles in a milk analog are stronger than the repulsive forces. Typically, aggregation is inhibited by the presence of a strong electrostatic repulsion due to electrical charges on the surfaces of the particles. However, these repulsive forces may be weakened if the pH or ionic strength is changed. The nature of the interactions between the colloidal particles in milk analogs depends on their composition, especially the nature of the molecules at their surfaces. For instance, the charge on proteins tends to go from positive to negative when the pH is increased from below to above their

TABLE 8 Summary of different kinds of colloidal interactions that can act between the particles (oil bodies, fat droplets, protein aggregates, or plant tissue fragments) in milk analogs

Interaction	Origin	Sign	Strength	Range
Van der Waals	Random fluctuations of partial charges	Attractive	Strong	Long
Hydrophobic	Exposed non-polar patches on particle surfaces	Attractive	Strong	Long
Depletion	Non-adsorbed polymers	Attractive	Weak-strong (depends on polymer concentration)	Short
Bridging	Polymers that adsorb to multiple particles	Attractive	Strong	Short
Steric	Adsorbed polymer layers	Repulsive	Strong	Short
Electrostatic	Charged surfaces	Repulsive or attractive	Weak-strong (depends on charge and ionic strength)	Short-long(depends on ionic strength)

isoelectric point (McClements et al., 2017). Around the isoelectric point, protein-coated particles tend to aggregate due to the weakening of the electrostatic repulsion between them. Phospholipids often have a quite high negative charge under neutral conditions, but the magnitude of this charge decreases when the solution becomes more acidic, which can again lead to aggregation due to a reduction in electrostatic repulsion. Adding mineral ions, particularly multivalent counter-ions, to a milk analog can also promote particle aggregation by electrostatic screening or ion binding effects. Consequently, it is important to establish the nature of the colloidal particles in a plant-based milk analog and how their surface characteristics (particularly charge and hydrophobicity) change with environmental conditions such as pH, ionic strength, and temperature. In general, the colloidal interactions between the particles in plant-based milk analogs can be described by the following expression (McClements, 2015):

$$W(h)_{\text{Total}} = W(h)_{\text{VDW}} + W(h)_{\text{ES}} + W(h)_{\text{S}} + W(h)_{\text{H}} + W(h)_{\text{D}} + W(h)_{\text{B}} \quad (10)$$

Here, $W(h)$ is the colloidal interaction potential, which is a measure of the free energy it takes to bring two particles to a surface-to-surface separation of h. The subscripts refer to the different kinds of colloidal interactions that can act between the particles, including van der Waals (VDW), electrostatic (ES), steric (S), hydrophobic (H), depletion (D) and bridging (B). Information about the origin, sign, range, and strength of these interactions is included in Table 8. Typically, the colloidal particles in a milk analog will aggregate when the sum of the attractive forces exceeds the sum of the repulsive forces. Thus, to ensure good resistance to particle aggregation it is important to increase the magnitude of any repulsive interactions and/or decrease the magnitude of any attractive interactions. This requires identifying the most important colloidal interactions operating in the milk analog.

The tendency for the colloidal particles in some milk analogs to aggregate can lead to various undesirable alterations in their perceived quality attributes: (i) large aggregates may cream or sediment faster (see next paragraph); (ii) sufficiently large aggregates may become visible to the human eye, leading to an undesirable appearance; (iii) sufficiently large aggregates may be perceived as gritty or grainy in the mouth, leading to an unpleasant mouthfeel. Consequently, it is important to avoid extensive particle aggregation in plant-based milk analogs.

The other major instability mechanism in milk analogs is gravitational separation, which involves the upward movement of particles because they have a lower density than the surrounding aqueous phase (creaming) or the downward movement of particles because they have a higher density (sedimentation). Oil bodies and fat droplets tend to move upward because they are lighter than water, whereas protein aggregates and plant tissue fragments tend to move downward because they are heavier. To a first approximation, the stability of a milk analog to gravitational separation can be described by Stokes' law:

$$v = -\frac{2gD^2(\rho_2 - \rho_1)}{18\eta_1} \quad (11)$$

Here, v is the velocity at which the particles move (+ upward/− downward), g is the gravitational acceleration constant, D is the particle diameter, ρ_2 is the particle density, ρ_1 is the aqueous phase density, and η_1 is the aqueous phase viscosity. Stokes' law predicts that the creaming stability increases when the particle diameter decreases, the density contrast decreases, or the aqueous phase viscosity increases. This gives manufacturers of milk analogs a

number of strategies for improving the resistance of their products to separation due to gravity. It is difficult to change the density contrast but creaming or sedimentation can be inhibited by reducing the particle size (e.g., by homogenization) or by increasing the aqueous phase viscosity (e.g., by adding thickening agents).

The taste, aroma, and mouthfeel of milk analogs depend on their unique compositions and structures. Many milk analogs contain flavors from the plant materials they were prepared from. As an example, soy milks have a beany flavor whereas hazelnut milks have a nutty flavor (Sethi et al., 2016). Moreover, some plant proteins have undesirable off flavors, such as astringent, earthy, or vegetative notes (Watson, 2019). Consequently, there is a great deal of research on trying to reduce the presence of these undesirable flavor notes using plant-breeding or processing methods.

Numerous studies have demonstrated that milk analogs may exhibit instability when used in certain food applications. For instance, milk analogs containing plant protein-coated oil droplets may aggregate around pH 5 because of a reduction in their charge (Gumus, Decker, & McClements, 2017a), which makes them unsuitable for applications as non-dairy coffee creamers because hot coffee has a pH of around this value (Chung, Sher, Rousset, & McClements, 2018). Researchers have also shown that there are large differences in the storage stability of commercial milk analogs due to gravitational separation (Jeske, Zannini, & Arendt, 2017), which can mainly be attributed to differences in their particle size and viscosity. Products containing smaller particles or with higher viscosities should be more resistant to separation. Coconut milk has been shown to be unstable to heating, high salt levels, and pH values near the isoelectric point, which was attributed to increased particle aggregation (Patil & Benjakul, 2018). Other milk analogs have also been reported to exhibit a similar stability profile, including those stabilized by soy proteins (Euston, Al-Bakkush, & Campbell, 2009; Ryan, McEvoy, McSweeney, O'Callaghan, & FitzGerald, 2008), faba bean, pea, and lentil proteins (Gumus et al., 2017a). Moreover, in milk analogs that contain polyunsaturated lipids (such as flaxseed oil) it is important to design the system to inhibit their oxidation during storage and processing, so as to avoid rancidity (Gumus, Decker, & McClements, 2017b). As a conclusion, it is important to identify the physical and chemical instability mechanisms operating in each product and then carefully control the composition and structure of the system to inhibit these processes.

5.7.4 | Nutritional, sustainability, and ethical comparison

The nutritional profiles of a number of plant-based milk analogs are compared to that of cow's milk in Table 9. There are substantial variations in the nutrient compositions of the different kinds of milk analogs, as well between them and the real milk. With the exception of soymilk, the milk analogs have a lower protein content than real milk. The milk analogs have a lower total and saturated fat content than whole milk, but higher than skim milk. The milk analogs contain no cholesterol, whereas the real milk does. The sugar contents of the milk analogs are less than those of cow's milk, but they mainly contain sucrose whereas the cow's milk contains lactose. All of the milk analogs contain higher calcium levels than real milk because they are fortified with calcium carbonate. They are also fortified with a number of oil-soluble vitamins, including vitamin A, D, and E. It should be noted that there are a variety of different kinds of milk analog in each category (e.g., original, sweetened, flavored, and reduced calorie versions of soymilk), which affects their nutritional profiles. As discussed earlier for other plant-based foods, milk analogs will have different metabolic and physiological responses than bovine milk, which go beyond simple differences in their macronutrient composition, due to their different rates and extents of digestion and absorption. Overall, the reported differences in nutritional profiles of milk analogs and milks may have some impact on human health, but at present this is unknown and will depend on the type and quantity of products consumed.

The potential environmental impact of consuming plant-based milk analogs rather than cow's milk is highlighted in Table 10 (Poore & Nemecek, 2018). The results show that there is much less land used, water use, greenhouse gas emissions, and pollution caused by the production of milk analogs than real milk. For instance, for soymilk, there was 93% less land used, 96% less water use, 69% less greenhouse gas emissions, and 90% less eutrophication compared to real milk. Additionally, the United Nations FAO estimates there are around 264 million dairy cows worldwide, which are used for the production of milk and dairy products. There are concerns with animal welfare when these cows are mainly housed indoors under conditions that are inconsistent with their natural tendencies as grazing animals (Knaus, 2016). These findings indicate that there are major environmental and ethical benefits from introducing more plant-based milk analogs into consumers' diets.

TABLE 9 Comparison of nutrient content of bovine milk and plant-based milk analogs

	Whole milk	Skim milk	Almond	Soy	Oat	Coconut
Calories per 240 mL serving (kcal)	150	80	60	110	90	70
	Percentage content (w/w)					
Total Fat	3.3	0.0	1.0	1.9	1.5	1.9
Saturated fat	2.1	0.0	0.0	0.2	0.0	1.7
Trans fat	0.0	0.0	0.0	0.0	0.0	0.0
Polyunsaturated fat	0.0	0.0	0.2	1.0	0.0	0.0
Monounsaturated fat	0.0	0.0	0.6	0.4	0.8	0.0
Cholesterol	0.015	0.002	0.0	0.0	0.0	0.0
Sodium	0.052	0.052	0.063	0.038	0.040	0.027
Calcium	0.125	0.129	0.188	0.188	0.192	0.192
Total carbohydrate	5.0	5.4	3.3	3.8	5.4	2.1
Dietary fiber	0.0	0.0	0.0	0.8	0.0	0.0
Sugars	5.0	5.0	2.9	2.5	2.1	2.1
Protein	3.3	3.3	0.4	3.3	0.4	0.0

Information taken from Silk foods (https://silk.com/plant-based-products/) and the USDA FoodData Central database (https://fdc.nal.usda.gov/).

Note: Values are given per 100 g of product but calories are given per serving size (240 mL). There will be appreciable differences in these values depending on the origin of the animal and plant-based products compared.

Ingredients: Almond milk (filtered water, almonds), cane sugar, vitamin and mineral blend (calcium carbonate, vitamin E acetate, vitamin A palmitate, vitamin D2), sea salt, gellan gum, sunflower lecithin, locust bean gum, ascorbic acid (to protect freshness), natural flavor.

Soymilk (filtered water, soybeans), cane sugar, vitamin and mineral blend (tricalcium phosphate, calcium carbonate, vitamin A palmitate, vitamin D2, riboflavin [B2], vitamin B12), sea salt, natural flavor, gellan gum.

Coconut milk (filtered water, coconut cream), cane sugar, vitamin and mineral blend (calcium carbonate, vitamin E acetate, vitamin A palmitate, vitamin D2, vitamin B12), dipotassium phosphate, sea salt, sunflower lecithin, gellan gum, locust bean gum, ascorbic acid (to protect freshness), natural flavor.

Oat milk (filtered water, oat concentrate), sunflower oil, vitamin and mineral blend (calcium carbonate, vitamin A palmitate, vitamin D2, riboflavin [B2], vitamin B12), dipotassium phosphate, sea salt, gellan gum, ascorbic acid (to protect freshness).

whole milk: milk and vitamin D_3.

Skim milk: Fat free milk, vitamin D palmitate, and vitamin D_3.

TABLE 10 Comparison of environmental impact of plant-based milk analogs and cow's milk

Impact category	Units	Cow's milk	Soy milk
GHG emissions	kg CO_2eq	3.2	1.0
Eutrophication	g PO_4^{3-}eq	10.7	1.1
Land use	m^2	9.0	0.7
Water use	L	628	28

Data obtained from Poore, J., & Nemecek, T. (2018).

5.8 | Other nutritional aspects

The nutritional implications of consuming plant-based foods were briefly addressed in the previous sections by comparing their nutritional labels with those of the animal-derived foods they were designed to replace. However, there are also numerous other nutritional aspects that need to be considered. As well as having different nutrient compositions, the kinetics of nutrient digestion and absorption may be different, which would influence the rate that sugars, lipids, and peptides enter the bloodstream after food consumption. As a result, this could impact a person's metabolic and satiety responses to foods, thereby influencing their susceptibility to chronic diseases such as diabetes and obesity. Moreover, there are differences in macronutrient and micronutrient bioavailability between plant-based and animal-based foods. For instance, many plant proteins are less digestible than animal ones because of the presence of phytates, and so they may provide less energy and essential amino acids to the human diet (Gibson, Bailey, Gibbs, & Ferguson, 2010; Gibson, Raboy, & King, 2018). These problems can often be overcome using appropriate processing operations but they should be carefully accounted for during product development. As mentioned earlier, it is also important to obtain a good balance of essential amino acids in plant-based food products, which often requires combining two or more different plant protein sources with complementary amino acid profiles (van Vliet, Burd, & van Loon, 2015). In addition, essential minerals, such as iron and calcium, are often more bioavailable in animal-based foods than their plant-based analogs (Antoine et al., 2021; Rousseau, Kyomugasho, Celus, Hendrickx, & Grauwet, 2020). The current generation of plant-based foods should therefore not be seen as being nutritionally equivalent to animal-derived foods (van Vliet, Kronberg, & Provenza, 2020). Clearly, further research is needed to establish the nutritional differences between specific plant-based and animal-based foods, and to develop effective strategies to overcome any potential nutritional deficiencies. In addition, it is important to consider the potential of inducing allergic reactions in some people when formulating foods with new kinds of proteins.

5.9 | Marketing and regulatory aspects

There are a number of issues impacting the marketing and regulation of plant-based food products that also need to be addressed when developing these kinds of novel foods. At present, many plant-based food analogs contain a large number of different ingredients, some of which may not be considered favorable to consumers (such as titanium dioxide, iron oxide, or methyl cellulose) (Alcorta, Porta, Tarrega, Alvarez, & Vaquero, 2021). Consequently, many plant-based food companies are trying to reformulate their products so as to reduce the total number of ingredients they contain, as well as to only use natural ingredients that consumers perceive as being label friendly. Another issue that needs to be addressed is related to the naming of plant-based food products. In some countries, regulators are not permitting companies to use terms such as "meat," "milk," or "egg" on plant-based food analogs, arguing that these terms should be reserved for the real animal-derived products (Alcorta et al., 2021; Aschemann-Witzel, Gantriis, Fraga, & Perez-Cueto, 2020). Much of the opposition to the use of these terms has come from the traditional farming industry whose livelihoods and markets are threatened by the increasing popularity of plant-based foods. Currently, the regulations governing the labeling of plant-based foods vary from region to region, which should be considered when developing and marketing these products.

Finally, there is growing interest in the development of hybrid products that contain a mixture of animal- and plant-derived ingredients (such as meat combined with mushrooms) for the flexitarian market (Grasso & Jaworska, 2020). These hybrid products aim to reduce the total quantity of animal-derived products in the human diet, thereby giving environmental and sustainability benefits. However, by only replacing some of the animal ingredients with plant-based ones there may also be improvements in the sensory properties and healthiness of hybrid foods, thereby leading to greater consumer acceptance. As a result, these kinds of products could have a major impact on improving the sustainability of the modern food supply as most consumers are neither vegans nor vegetarians.

6 | CONCLUSIONS AND FUTURE DIRECTIONS

The transformation to a more plant-based diet has been strongly encouraged by environmental and health experts to create a food supply that will feed the growing global population without causing irreparable damage to the planet. As a result, an increasing number of consumers are changing their eating habits to adopt a more healthy, sustainable, and ethical plant-based diet, which has led to a rapid growth in plant-based foods. Nevertheless, there are still a number of hurdles that need to be overcome before a greater proportion of the population adopt this kind of diet:

- Consumer hurdles: There is a lack of understanding of consumer perceptions toward different kinds of plant-based foods and of the factors that impact their consumption. This knowledge is required to develop effective strategies to encourage consumers to try, accept, and adopt these products.
- Technological hurdles: There is a lack of high-quality plant-based ingredients with the required functional attributes, as well as a lack of suitable processing technologies to convert these ingredients into plant-based foods that consumers actually want to eat. This is particularly true for products with complex structural hierarchies such as plant-based alternatives to whole muscle meat, fish, and cheese.
- Commercialization hurdles: There is a lack of information available to both small and large companies about the commercial landscape of plant-based foods, including the affordability and relative costs of different ingredients and processes, regulatory frameworks, supply chain issues, and safety concerns. The availability of this knowledge would facilitate the entry of companies into the plant-based food market.
- Socio-economic hurdles: There is a lack of knowledge about the social, economic, health, and environmental impact of replacing animal products with plant-based alternatives in the human diet. This information would help governments draft policies that would foster the transition to a more healthy and sustainable diet, while promoting economic development, wealth creation and employment.
- Nutritional, safety, and health hurdles: There is also a lack of knowledge about the nutritional, safety, and health implications of switching from an omnivore diet to a fully plant-based one. In particular, more research is required on the impact of differences in protein digestion, absorption, amino acid profiles, and allergenicity on human health and wellbeing.

Progress in this area will therefore depend on taking an integrated multidisciplinary approach to overcome these hurdles, which will involve the work of agricultural scientists, food scientists, nutritionists, engineers, social scientists, psychologists, economists, and environmental scientists. Increased government, industry, and philanthropic funding in this area is needed to support this work and therefore facilitate the transition to a more sustainable and healthy plant-based diet.

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AUTHOR CONTRIBUTIONS

David Julian McClements—Conceptualization; data curation; funding acquisition; project administration; resources; writing-original draft; writing-review & editing. Lutz Grossmann: Conceptualization; writing-original draft; writing-review and editing.

CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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