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Food Proteins: Technological, Nutritional, and Sustainability Attributes of Traditional and Emerging Proteins

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Keywords

protein, food, digestibility, sustainability, cultured meat, insect protein, plant protein, algal protein

Abstract

Protein is an essential macronutrient and a key structural component of many foods. The nutritional and technological properties of food protein ingredients depend on their source, extraction and purification, modification during food manufacture, and interactions with other food components. In addition to covering these elements, this review seeks to highlight underappreciated aspects of protein environmental sustainability and explores the potential of cultured meat and insect-derived proteins.

INTRODUCTION

Protein is a dietary macronutrient that plays numerous structural and functional roles in the body; without protein intake, we would die. In addition, protein-based ingredients fulfill many different technological roles in formulated foods and contribute to texture, color, flavor, and other properties. This review examines how protein-rich food ingredients are produced; how they are used in creating formulated foods, i.e., foods that are assembled or created from a combination of partially purified ingredients; and how the nutritional value of food proteins depends on source and processing.

Proteins are heterogeneous in composition, structure, and functionality. A single protein molecule may contain hydrophobic and hydrophilic regions, structured and unstructured regions, and positive, negative, and uncharged regions. Amino acid side-chains differ in their size, charge, and reactivity, and the biological importance of amino acids varies from essential or conditionally essential to nonessential.

A similar level of complexity applies equally to carbohydrates and lipids, and it presents both challenges and opportunities. Here, I describe how this complexity is manifested in the properties of high-protein food ingredients, and how it can be understood and utilized to formulate safe and delicious foods that also deliver high-quality protein nutrition.

A large research effort has gone into establishing how much protein we need to eat to remain healthy, and these guidelines are summarized in **Table 1**. Estimated Average Requirements (EARs) are the average daily intake level estimated to meet the requirements of half of the healthy individuals in a group, whereas Recommended Dietary Allowance (RDA) is the average daily dietary intake sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a group (Inst. Med. 2005). There is some debate about RDAs for adults over the age of 65, and

Table 1 Protein intake data by life stage, compiled from the Institute of Medicine (2005) except as indicated in footnotes

Life stage	Estimated average requirement (g/kg/d)[a]	Recommended dietary allowance (g/d)[b]	Reference weight (kg)
Children			
2–6 months	1.12[c]	9.1[d]	6
6–12 months	1.0	11.0	9
1–3 years	0.87	13	12
4–8 years	0.76	19	20
Men			
9–13 years	0.76	34	36
14–18 years	0.73	52	61
>18 years	0.66	56	70
Women			
9–13 years	0.76	34	37
14–18 years	0.71	46	54
>18 years	0.66	46	57
Pregnancy	0.88	71	57
Lactation	1.05	71	57

[a]Grams of protein per kilogram of body weight per day.
[b]Grams per day, based on reference body weights.
[c]From World Health Organization (2007).
[d]Adequate intake: mean intake for healthy breastfed infants.

recent evidence suggests that for this group, an intake on the order of 50% higher than the average adult RDA [i.e., an increase from 0.8 g/(kg·d) to 1.2 g/(kg·d)] is needed to compensate for an age-related decrease in physiological responsiveness to protein intake (Baum et al. 2016).

SOURCES OF FOOD PROTEIN

Food proteins come from a wide variety of sources (**Figure 1**). Animal proteins have been consumed for many millennia; plant proteins became more prevalent in the human diet as a result of advances in crop breeding and the Agricultural Revolution around 10,000 BCE.

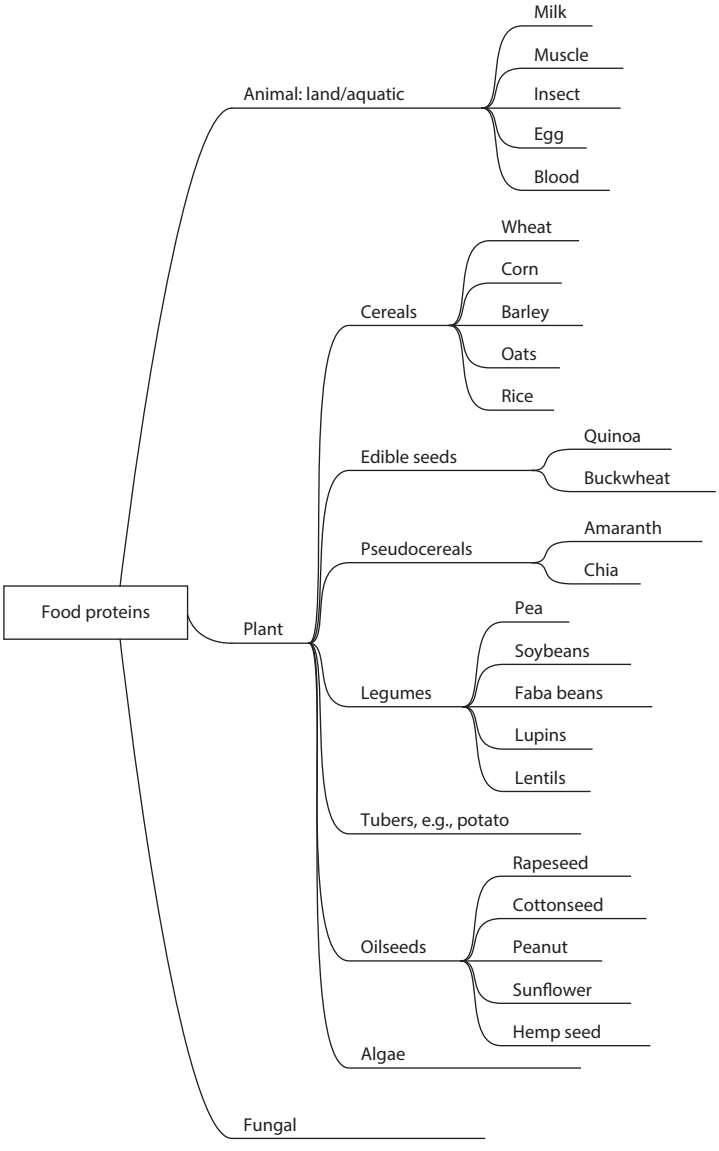

Figure 1

Major sources of food protein, classified according to origin.

Table 2 Summary of protein content by class in various plant-derived foods[a]

Plant source	Albumins (%)	Globulins (%)	Prolamins (%)	Glutelins (%)
Wheat	6–10	5–8	35–40	40
Rice	2–6	12	4	80
Barley	3–5	10–20	35–45	35–45
Maize	4	4	60	26
Sorghum	2–7	2–10	35–60	20–35
Soybean	NA	90	NA	NA
Pea	15–25	50–60	NA	NA
Chickpea	8–12	53–60	3–7	19–25
Lupin	25	75	NA	NA
Canola	20	60	2–5	15–20

[a]Adapted with permission from Day (2013).
Abbreviation: NA, not available.

The isolation of protein-rich fractions from foods is a relatively recent phenomenon, perhaps originating from spontaneous coagulation of animal blood or rennet-induced coagulation of milk in animal stomach pouches. By the end of the nineteenth century, there was a sophisticated understanding of protein fractionation, and this was expressed systematically in Thomas B. Osborne's system for classifying plant proteins (Osborne 1908):

- Albumins: soluble in water and susceptible to heat-coagulation
- Globulins: insoluble in water, soluble in dilute salt solution, e.g., 0.1 M NaCl
- Prolamins: insoluble in water, soluble in 70%–80% aqueous ethanol and heat-resistant
- Glutelins: insoluble in water, soluble in dilute alkali, e.g., 0.1 M NaOH solution

This empirical scheme is still in use today; each fraction contains a complex mixture of proteins and there is some overlap between classes, but it is nevertheless a useful starting point. **Table 2** summarizes the fractions in various plant proteins. It can be seen that legumes contain predominantly albumins and globulins, whereas cereal protein is dominated by poorly soluble prolamins and glutelins, which explains why nondairy milk substitutes made from cereals (and nuts) are very low in protein (Vanga & Raghavan 2018).

Albumins and globulins can be classified with ultracentrifugation-derived Svedberg sedimentation coefficients, which are a measure of hydrodynamic size and are expressed in Svedberg units (S). The albumins are predominantly 2S proteins, i.e., they have sedimentation coefficients distributed around a mode of 2 Svedbergs, whereas globulins occur in 7–8S and 11–12S groups (Häkkinen et al. 2018, Shewry & Casey 1999). Aggregated or insoluble proteins can be further chemically fractionated on the basis of solubility with concentrated urea, reducing agents, and/or detergents (Liu & Hsieh 2008). In biochemical disciplines plant proteins are sometimes classified on the basis of function: storage proteins, structural and metabolic proteins, or protective proteins (Shewry & Casey 1999).

The Osborne fractionation scheme was developed in the context of plant protein research, but similar principles are applied to the classification of meat proteins (Strasburg et al. 2007):

- Sarcoplasmic proteins: soluble in water at low ionic strength
- Myofibrillar proteins: soluble at high salt concentration, e.g., >0.3 M NaCl
- Stromal proteins: insoluble in water or salt solution

A knowledge of these fractions is particularly important to the manufacture of surimi (Park & Lin 2005). When fish meat is washed during the surimi process, the removal of sarcoplasmic proteins and the retention of myofibrillar proteins give the highest quality and yield. An ionic strength of 0.01–0.1 and pH 5.5 minimize the solubility of myofibrillar proteins and optimize their separation from sarcoplasmic proteins (Stefansson & Hultin 1994). A similar phenomenon occurs with meat proteins from land animals (Xiong 2014).

The functional properties of proteins can be modified by processing; for example, poorly soluble protein can be solubilized by acid-, alkali-, and/or heat-induced denaturation and hydrolysis, as in the conversion of insoluble collagen into soluble gelatin (Haug & Draget 2011). Heat-sensitive proteins such as whey protein can be enzymatically hydrolyzed to improve heat stability and reduce allergenicity (Butré et al. 2012, Kankanamge et al. 2015). Some protein sources are particularly heterogeneous because the entire organism is processed, e.g., mycoprotein, algal proteins, and insect-derived proteins. This heterogeneity is particularly evident in polyacrylamide gel electrophoresis (Yi et al. 2013).

PROTEIN PURIFICATION

Mammalian milk proteins are readily fractionated with acid or rennet (**Figure 2**). Rennet is an enzyme that hydrolyzes the κ-caseins that form a "hairy layer" on the surface of native casein micelles and thereby removes steric stabilizing forces, leading to self-association and precipitation of micelles. Acid destabilizes casein micelles by neutralizing the charges on surface κ-casein molecules so that they collapse onto the surface of micelles, which are thus destabilized. These phenomena are discussed by Dalgleish (2014).

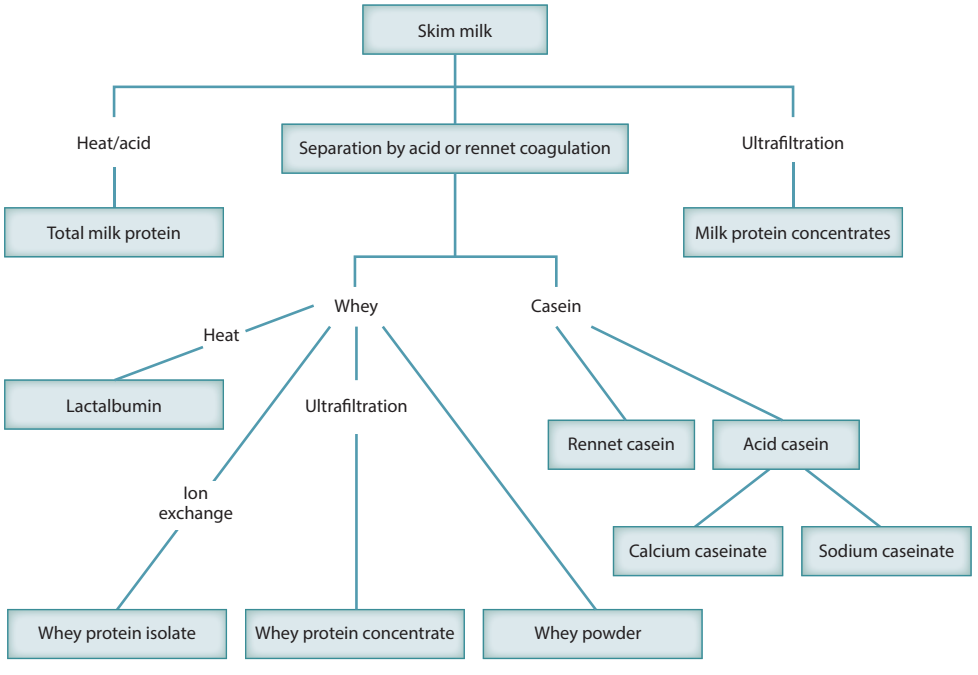

Figure 2

Industrial milk protein fractionation scheme. Adapted with permission from Singh (2011).

Native whey proteins are resistant to the action of rennet; at high enough concentration they precipitate close to their isoelectric point (pH 5.1), but they remain soluble under industrial acidification protocols, allowing separation by screening from caseins, which precipitate at pH ~4.6. The whey fraction thus produced can be purified further by removing lactose and minerals with ultrafiltration and/or ion exchange to produce whey protein concentrates (up to 80% w/w protein) or isolates (75–90% w/w protein). In **Figure 2**, "lactalbumin" refers to an insoluble powder produced in New Zealand from the 1950s onward by heat-precipitating whey proteins (Matthews 2014); the protein α-lactalbumin is the second most abundant component of bovine whey protein after β-lactoglobulin.

The extraction and purification of plant protein are more involved, which reflects the fact that plant proteins are often sequestered as insoluble, inert bodies within seeds. **Figure 3** depicts the extraction and purification of soy proteins. Extraction of defatted soy flakes with alkaline or alcoholic solutions produces soy protein concentrate, which has low solubility unless further processed

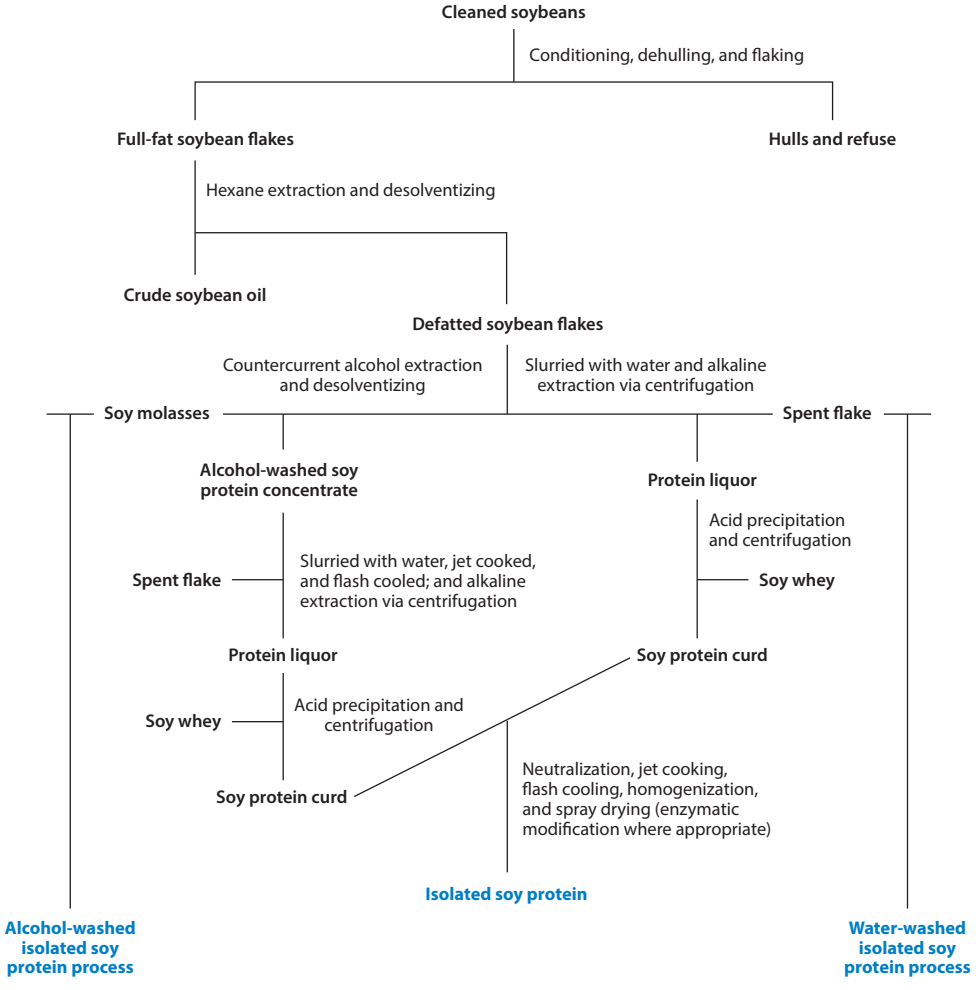

Figure 3

Isolated soy protein extraction and purification processes. Adapted from Egbert (2004).

by heating, homogenizing, and spray drying (Egbert 2004). The functional soy protein concentrates thus produced can gel, bind water, and emulsify fat (Nishinari et al. 2018). A subsequent acid precipitation step removes soluble carbohydrate impurities to produce soy protein isolates with a protein content of approximately 90% w/w (Egbert 2004).

Acid-precipitated soy protein isolate contains the major soybean storage proteins, but more than half of the protein in soy flake may be lost during processing, including a substantial amount in soy whey (Wu et al. 2014). Proteins can be recovered from soy whey by foam fractionation (Li et al. 2014) or ultrafiltration (Lassissi et al. 2014), and once heat-treated to eliminate antinutritional properties, soy whey proteins have good foaming and emulsifying activities (Feng et al. 2009, Ray & Rousseau 2013, Sobral et al. 2018).

FORMULATION WITH FOOD PROTEINS

The native structure of proteins is tailored to their function in the organism, whether this is acting as a nutrient reserve (seed storage proteins), delivering protein and minerals to the neonate (caseins), implementing mechanical support and movement (muscle proteins), carrying oxygen or vitamins (hemoglobin, lipocalins), or performing intracellular metabolic roles (enzymes, photosynthetic proteins) as well as other functions.

Protein extraction and food processing often involve denaturation of compact, structured proteins to activate useful functionalities, e.g., denaturation of globular whey proteins or egg proteins to improve gel-forming and emulsification. The caseins are unusual in being natively denatured, i.e., they have very little secondary structure in the native state (Horne 2009). These proteins are excellent emulsifiers and have high heat stability.

Extracting and purifying plant proteins often involve quite severe heat, shear, and/or solvent extraction processes, which inevitably lead to some degree of denaturation, cross-linking, and even hydrolysis. The kinetics of heat-induced protein denaturation depend on the heating temperature and the ionic environment, particularly pH and ionic strength (Loveday 2016).

Besides their nutritional roles, proteins play a wide variety of technological roles in foods (**Table 3**). Deliberate or incidental process-induced modifications to protein structure can have a significant impact on protein functionality, as shown for pea protein isolates in **Table 4**. The same is true of milk, meat, and egg protein ingredients, but to a lesser extent because of gentler processing. Moure et al. (2006) reported the functional properties of a wide range of oilseed proteins extracted under different conditions, and similar information for amaranth, quinoa, and chia was compiled by López et al. (2018). Antinutritional compounds found in oilseeds were discussed by Arntfield (2018).

At sufficiently high protein concentration, heat-denatured proteins can aggregate, particularly via hydrophobic interactions, hydrogen bonds, and disulfide bonds. The pattern of aggregation and subsequent gelation depends on solution conditions (**Figure 4**). Heating protein at low ionic strength and/or pH far from the isoelectric point (pI) leads to filamentous or fine-stranded aggregates with a string-of-beads morphology. Aggregation is more random at moderate or high ionic strengths ($\geq$50 mM NaCl or $\geq$10 mM $CaCl_2$) (Bryant & McClements 1998) or at pH approaching the pI, leading to particulate aggregates (Ako et al. 2009, Doi 1993).

Filamentous aggregates form physical entanglement networks with the addition of salt, which masks electrostatic repulsion. Hydrophobic interactions drive network formation in solutions of filamentous aggregates, but once gelled, other forces act to consolidate the network and increase gel strength (Bryant & McClements 1998). For that reason, gelling temperature strongly influences gel properties (Bryant & McClements 1998). These fine-stranded or "homogeneous" cold-set gels are typically transparent (Ako et al. 2009).

Table 3 Typical functionalities of proteins in food

Functionality	Example
Cross-linking	Enzyme, e.g., transglutaminase
	Heat-induced gelation
	Acid gelation
	Polyvalent ions
	Cold-set gels
Solubility	Heat stability in beverages
Emulsification	Conventional emulsions
	Pickering emulsions
Flavor/aroma	Meaty/roasted notes (cysteine)
Color	Maillard (nonenzymatic) browning
Antimicrobial	Lysozyme, lactoferrin
Texturization	Meat analogs
Foaming	Foaming capacity
	Foam stability
Water holding	Yogurt, processed meat

Particulate, or "heterogeneous," gels are more turbid and typically have a lower water-holding capacity. The transition between fine-stranded and particulate aggregation is very sensitive to pH, as shown in **Figure 5** (Langton & Hermansson 1992), and this reflects microphase separation phenomena (Ako et al. 2009). The rubberiness (fracture strain) of heat-set whey protein gels depends on the degree of disulfide bonding, whereas stiffness (modulus) reflects the degree of noncovalent associations within the gel (Havea et al. 2009). The physical chemistry of whey and other food protein gels was discussed at length by Ziegler & Foegeding (1990).

In a few cases, the native structure of a protein needs to be maintained in the food in order to deliver a biological functionality. The whey protein lactoferrin has heat-labile antimicrobial and antioxidative activities that relate to its ability to sequester metal ions (Korhonen & Marnila 2011). Hen egg white lysozyme also has antimicrobial activity (by a different mechanism) that relies on retention of the intact native structure (Strixner & Kulozik 2011).

PROTEIN DIGESTION

Proteins in food undergo physical and chemical changes as they pass through the mouth to the stomach, small intestine, and large intestine. Liquid beverages pass through the oral phase almost

Table 4 Functionalities of pea protein isolates produced from the CDC Striker cultivar by different extraction methods[a]

Extraction method	Water-holding capacity (g/g)	Oil-holding capacity (g/g)	Solubility (%)	Foaming capacity (%)	Foam stability (%)
AE-IP	2.4 ± 0.1	3.5 ± 0.2	64.1 ± 1.2	183.3 ± 0.0	68.0 ± 1.0
MP	3.5 ± 0.1	3.6 ± 0.2	42.8 ± 0.1	133.3 ± 0.0	77.8 ± 3.2
SE	0.3 ± 0.0	5.4 ± 0.1	91.1 ± 2.2	258.3 ± 11.8	48.9 ± 2.0

[a]Adapted with permission from Stone et al. (2015).
Abbreviations: AE-IP, alkali extraction–isoelectric precipitation; MP, micellar precipitation; SE, salt extraction.

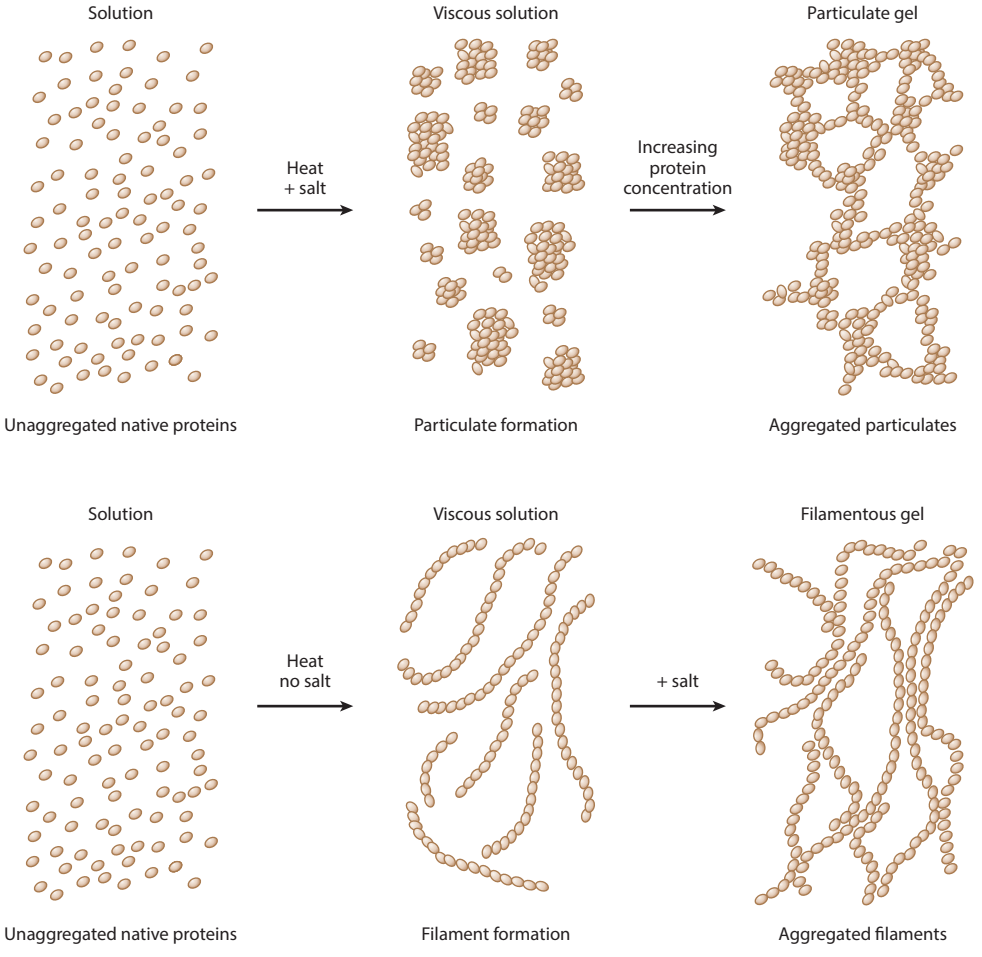

Figure 4

Development of particulate and fine-stranded (filamentous) protein gel structures. Adapted with permission from Bryant & McClements (1998).

unchanged, but solid or semisolid foods are crunched, chewed, sucked, or smooshed (Jeltema et al. 2016) in the mouth and combined with saliva. Saliva is ~99% water but also contains mucins, proline-rich proteins, amylase enzymes, and electrolytes (Mosca & Chen 2017). Salivary mucins can interact with protein-stabilized emulsions in the mouth to flocculate emulsion droplets, especially when their surface charge is positive at the pH of saliva, which is close to neutral (Mackie & Macierzanka 2010, Sarkar et al. 2009).

The major chemical changes in the stomach and small intestine are indicated in **Figure 6**. In the stomach, proteins are exposed to acid and the proteolytic enzyme pepsin, and the stomach contents (chyme) are gently mixed by peristaltic waves caused by contraction of the stomach wall muscles (Bornhorst 2017). The acidic pH denatures some proteins, which makes them susceptible to proteolysis by pepsin. The bovine whey protein β-lactoglobulin is extremely acid- and pepsin-resistant in its native form, but the heat-denatured form is rapidly hydrolyzed by pepsin (Peram et al. 2013), and even the native form is rapidly hydrolyzed by intestinal proteases.

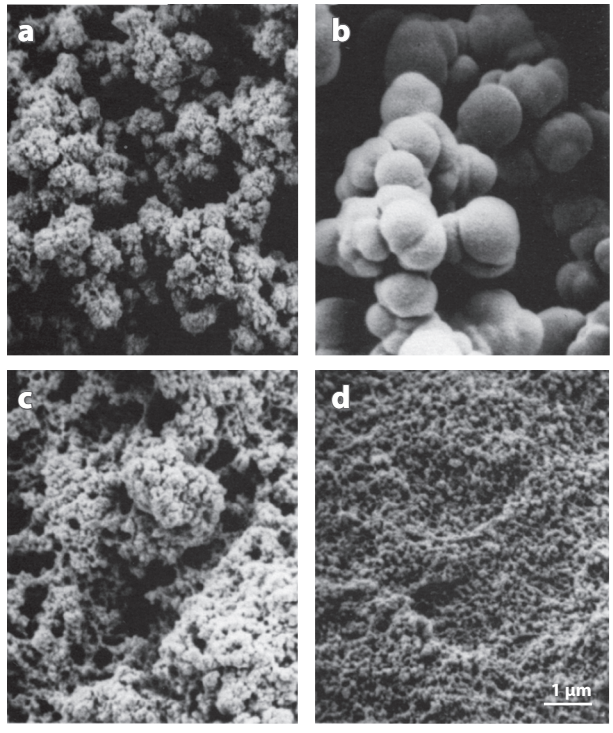

Figure 5

Scanning electron micrographs of heat-set β-lactoglobulin gels formed at different pH. (*a*) pH 6. (*b*) pH 5.5. (*c*) pH 4.5. (*d*) pH 4. Adapted with permission from Langton & Hermansson (1992).

The caseins appear to be unique in their ability to form a coagulum in the stomach when consumed as the micellar form that prevails in milk. The pH of chyme in the proximal stomach can remain above 6 for up to an hour after a meal due to the buffering effect of meal components (Bornhorst et al. 2014). Under these conditions, casein coagulation is driven by the cleavage of κ-caseins by pepsin (Ye et al. 2016), which removes steric stabilization. Once the pH of chyme drops below 5, even partially micellar forms of casein will coagulate somewhat due to the loss of electrostatic repulsion at acidic pH. This applies to sodium caseinate and casein complexes with whey or fat globules in heated/homogenized milk (Ye et al. 2016, 2017).

Gastric coagulation inhibits pepsinolysis by slowing the diffusion of pepsin into coagula (Thévenot et al. 2017), which slows the release of proteins/peptides into the small intestine and ultimately slows amino acid absorption. This effect creates a prolonged feeling of fullness, which contributes to appetite control, and a sustained supply of amino acids. Similar effects can be produced by structuring dairy products to either promote or delay pepsinolysis (Dupont et al. 2018).

As chyme exits the stomach into the duodenum, it encounters the hydrolytic enzymes trypsin and chymotrypsin, as well as alkaline pancreatic secretions that largely neutralize the pH. Intestinal proteolysis is remarkably effective, and most proteins are reduced to di- or tripeptides. However, the appearance in the bloodstream of diet-derived allergenic peptides (Wickham et al. 2009) and bioactive peptides from food proteins and gastrointestinal secretions (Dave et al. 2016, Moughan et al. 2014) shows that some peptides are partially digestion-resistant and can be absorbed. Even bioactive peptides that are not absorbed can modulate gastrointestinal function

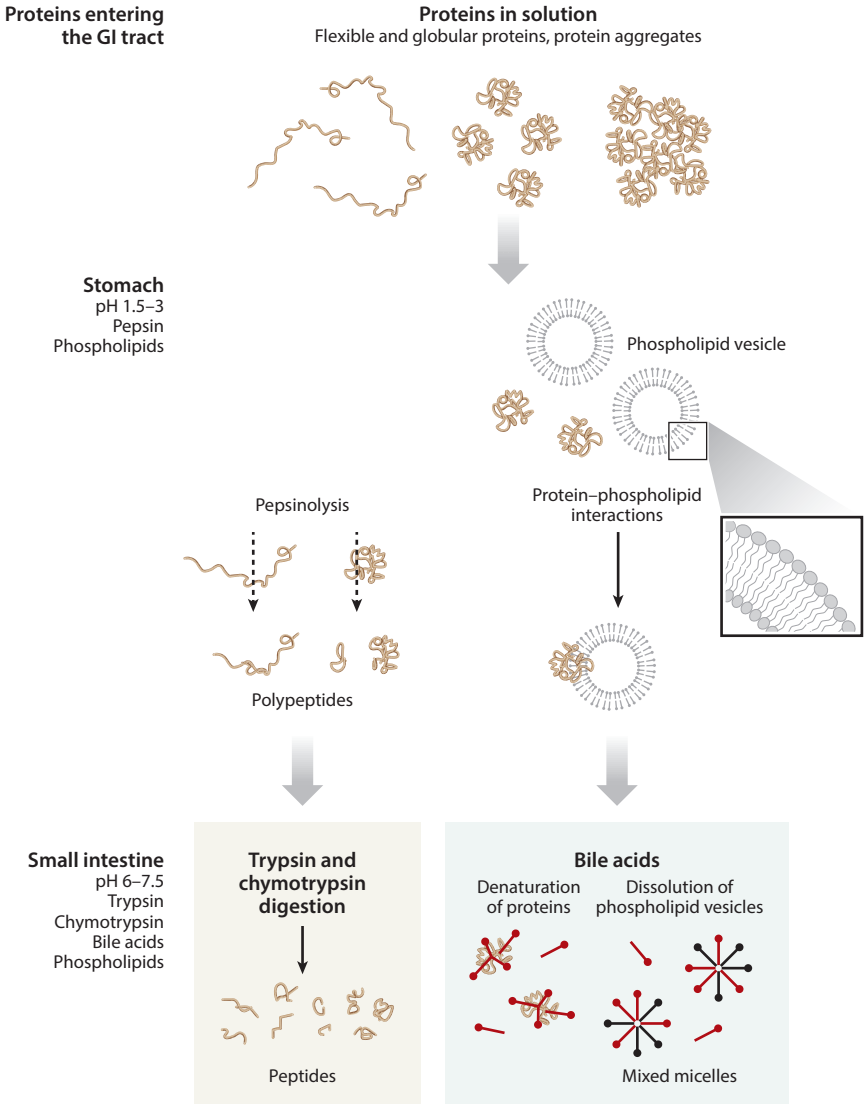

Figure 6

Schematic illustration of protein digestion processes at various stages of the gastrointestinal (GI) tract. Adapted with permission from Mackie & Macierzanka (2010).

and modify the digestion/absorption of macronutrients in metabolically significant ways (Shimizu 2004).

Proteins, peptides, and amino acids that have not been absorbed in the upper gastrointestinal tract can act as substrates for gastrointestinal microbiota. Microbes are most numerous in the colon and lower ileum, but they occur throughout the gastrointestinal tract. The metabolism of dietary proteins by gut microbiota may produce metabolites that are harmful to the host, and this is an area under active investigation (Lancha et al. 2017).

NUTRITIONAL QUALITY OF PROTEIN-RICH FOODS

Protein-rich foods vary in their nutritional quality, i.e., their ability to meet human requirements for amino acids. The factors influencing nutritional quality include protein content, proportions of different amino acids, and digestibility. Protein content can be measured with a variety of different assays (see **Table 5** and sidebar titled Protein Quantification). Intriguingly, the nutritional benefit of consuming protein exceeds that of consuming the constituent essential amino acids in corresponding quantities (Katsanos et al. 2008).

Digestibility is a measure of how well a human or animal can digest and absorb amino acids from dietary protein sources. Digestibility is specific to a given food material or ingredient rather than protein source because of variation in the physical and chemical availability of protein to digestive/absorptive processes and the co-occurrence of substances that may inhibit digestion and/or absorption. For these reasons, processing can either increase or decrease digestibility (Salazar-Villanea et al. 2016); e.g., autoclaving faba bean decreases protein digestibility in rats by 30% (Carbonaro et al. 2000), whereas extruding soya bean flakes increases digestibility by 18% (Aslaksen et al. 2006).

Lysine is particularly susceptible to process-induced chemical modification, which results in loss of bioavailability (Salazar-Villanea et al. 2016). Acid hydrolysis is a common precursor of amino acid quantification. The products of lysine modification are often acid-labile, which means

PROTEIN QUANTIFICATION

Protein content can be measured with a range of different assays (see **Table 5**). Assay selection considerations include a method's suitability for use with a given food material, its linear concentration range, the time and cost of running each assay, and the required level of accuracy (Moore et al. 2010). Certain solvents, detergents, chelators, and reducing agents can interfere with dye-binding methods (Noble & Bailey 2009). Several assays are sensitive to amino acid sequences, particularly those measuring aromatic amino acids (A_{280}), and protein standards should be selected with care (Moore et al. 2010, Noble & Bailey 2009). The Kjeldahl and Dumas methods require material-specific nitrogen conversion factors, reflecting different amino acid makeup. The calculation of conversion factors has substantial economic and environmental ramifications and is not without controversy (Int. Dairy Fed. 2016). The protein measurement standard for foods (CXS 234-1999) is maintained by the Codex Alimentarius Commission Committee on Methods of Analysis and Sampling (Codex Aliment. Comm. 2018).

Table 5 Overview of assays used to quantify protein content in food samples

Principle	Examples	Reference
Spectroscopy	Absorbance at 280 nm (tyrosine, tryptophan)	Noble 2014
	Absorbance at 205 nm (peptide bond)	
	Mid-infrared spectroscopy	De Marchi et al. 2014
	Raman spectroscopy	McGoverin et al. 2010
Dye binding	Bicinchoninic acid (BCA)	Walker 2009
	Biuret reaction with alkaline copper: Lowry and Folin-Ciocalteu assays	Waterborg 2009
	Coomassie blue: Bradford assay	Kruger 2009, Noble 2014
	Fluorescent free amine reagents: *o*-phthaldialdehyde, fluorescamine	
	Fluorescent interfacial probes: NanoOrange™, Quant-iT™	
Nitrogen content	Digestion, distillation, and ammonia determination: Kjeldahl method	Sáez-Plaza et al. 2013
	Combustion and N_2 determination: Dumas method	

that special analytical procedures are required to distinguish them from unmodified lysine; otherwise, bioavailable lysine will be overestimated (Rutherfurd & Moughan 2007).

Despite a range of sophisticated systems for simulating digestion in vitro (Verhoeckx et al. 2015), digestibility coefficients measured in vitro are indicative at best; in vivo measurements with animal models are more meaningful at present (FAO 2013). True ileal amino acid digestibility (TIAAD) is measured via the disappearance of dietary amino acids from the digestive tract, as measured at the terminal ileum (Wolfe et al. 2016) and corrected for endogenous ileal amino acids (Moughan & Rutherfurd 2012). Protein digestibility ranges for various food materials are summarized in **Figure 7**. Digestibility data for 10 food ingredients and 11 foods commonly consumed in India can be found in Rutherfurd et al. (2012), and meat protein digestibilities were reported by Cui et al. 2013.

Of the 20 amino acids utilized in human metabolism, 9 are considered essential or indispensable (which appear to mean the same thing) because they cannot be synthesized by the body: leucine, isoleucine, valine, lysine, threonine, tryptophan, methionine, phenylalanine, and histidine. They must be consumed as part of the diet, and the quantity of digestible indispensable amino acids therefore limits the nutritional value of food protein. This is quantified as the Digestible Indispensable Amino Acid Score (DIAAS) (Wolfe et al. 2016), which is calculated as follows:

$$\begin{aligned}\text{DIAAS} &= 100 \times \frac{\text{mg of digestible IAA in 1g of the test food}}{\text{mg of the same amino acid in 1g of the reference food}} \\ &= 100 \times \frac{\text{TIAAD for test protein} \times \text{amino acid content of test food}}{\text{TIAAD for reference protein} \times \text{amino acid content of reference food}}.\end{aligned}$$

Reference values in the denominator are calculated from age-specific amino acid requirement patterns and EARs for protein intake (Wolfe et al. 2016). Consequently, DIAAS values are slightly different for infants, children, adults, and seniors. The DIAAS of the first limiting indispensable amino acid (i.e., lowest DIAAS) can be considered the overall DIAAS for the test food. **Table 6** shows DIAAS values for a range of foodstuffs. Nutritional deficiencies of individual protein sources can be overcome by combining them with foods having complementary amino acid digestibility.

Combining corn-based breakfast cereal with bovine milk overcomes low lysine digestibility of corn protein (DIAAS of 0.012) to raise overall DIAAS to 1.07 (Rutherfurd et al. 2015). An algorithm for matching plant-based foods on the basis of complementary amino acid content was developed by Woolf et al. (2011) and made available on a website called vProtein (**http://www.vprotein.com**). Although vProtein does not account for digestibility, it demonstrates the potential to automate dietary selection algorithms.

DIAAS has been endorsed by the Food and Agriculture Organization of the United Nations as the gold standard method for protein nutritional quality (FAO 2013, Moughan et al. 2012). A current barrier to widespread use of DIAAS is the limited amount of data available; Massey University in New Zealand and Wageningen University and Research in the Netherlands are working to rectify this through the Proteos collaboration.

SUSTAINABILITY OF FOOD PROTEINS

The sustainability of foods is a technically complex and politically charged topic. Even defining the system boundaries within which sustainability is assessed is difficult to do objectively.

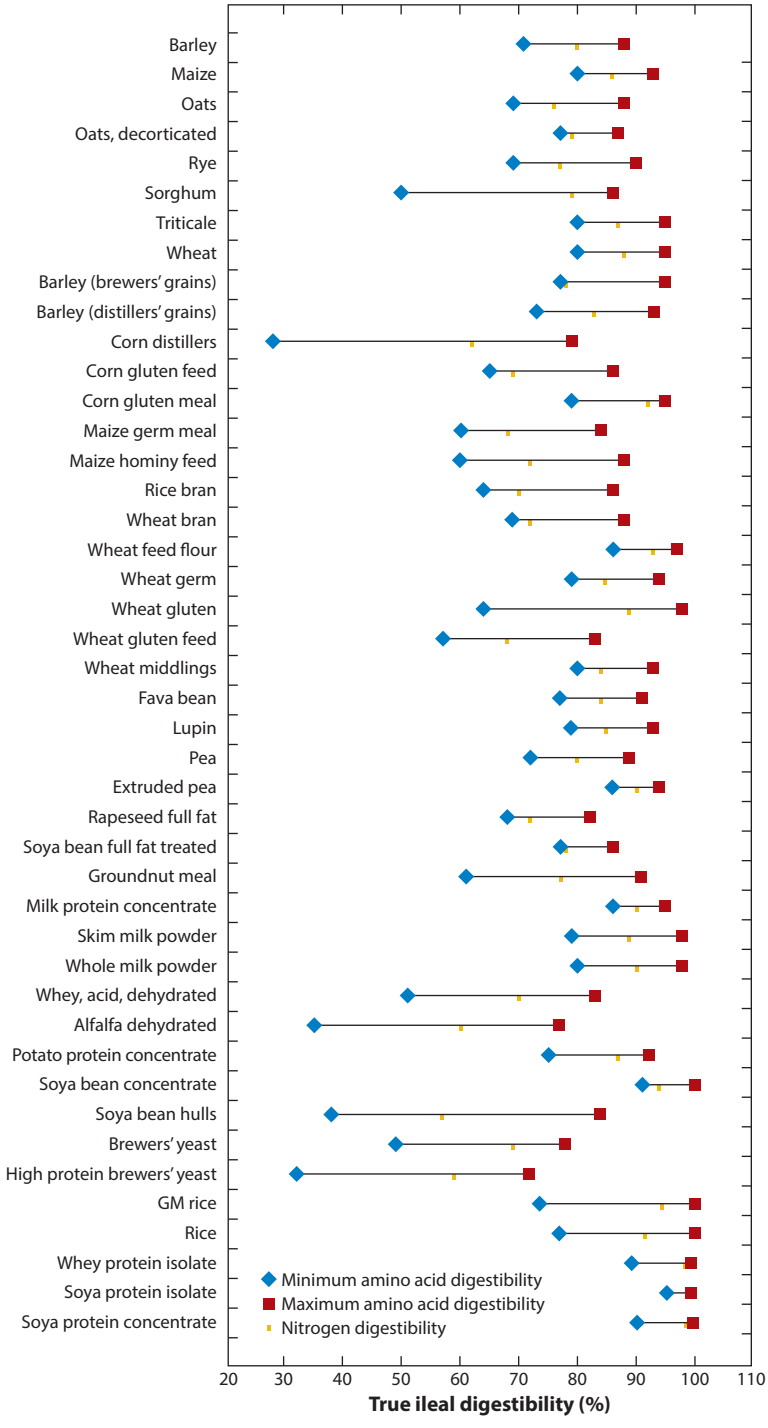

Figure 7

Maximum true ileal amino acid digestibility (*red squares*), minimum true ileal amino acid digestibility (*blue diamonds*), and true ileal nitrogen digestibility (*gold tick marks*) of amino acids in protein-rich foods and feeds. Adapted with permission from Moughan et al. (2012). Abbreviation: GM, genetically modified.

Table 6 Digestible indispensable amino acid scores (DIAAS) for 14 protein-rich foods as measured in growing male rats[a]

Food material	DIAAS	Limiting amino acid
Milk protein concentrate	1.18	Methionine + cysteine
Whey protein isolate	1.09	Histidine
Whey protein concentrate	0.973	Histidine
Soy protein isolate A	0.898	Methionine + cysteine
Soy protein isolate B	0.906	Methionine + cysteine
Pea protein concentrate	0.822	Methionine + cysteine
Cooked peas	0.579	Methionine + cysteine
Cooked kidney beans	0.588	Methionine + cysteine
Cooked rice	0.595	Lysine
Cooked rolled oats	0.542	Lysine
Wheat bran	0.411	Lysine
Roasted peanuts	0.434	Lysine
Rice protein concentrate	0.371	Lysine
Corn-based breakfast cereal without milk	0.012	Lysine
Corn-based breakfast cereal with milk	1.07	Lysine

[a]Adapted with permission from Rutherfurd et al. (2015).

Sustainability can be viewed in terms of footprinting for greenhouse gases, water, energy, social impact, distance to market, or other variables; there is currently no consensus about what sustainability is. The objective of this section is to raise a few often-overlooked factors contributing to our understanding of the environmental impact of food protein production.

The nutritional context within which protein is placed influences sustainability calculations. Protein can be metabolized for energy, which could otherwise come from carbohydrates or fats. However, viewing protein as an energy source undervalues its role in supplying indispensable amino acids, a role that cannot be performed by other nutrients. If dietary protein is viewed primarily as a source of indispensable amino acids, then the quality of a given protein-rich food as a source of digestible indispensable amino acids (the DIAAS) is important.

A recent high-profile study by Poore & Nemecek (2018) attempted a quantitative comparison of environmental impacts among a range of different foodstuffs. The protein-rich foods were compared on a "per 100 g protein" basis for solid foods or a "per L standardized at 3.3% protein" basis for bovine and soy milk. This comparison failed to recognize the large difference in protein quality (i.e., DIAAS) between different protein sources, which is known to significantly affect land use comparisons (Ertl et al. 2016). The effect of this omission is illustrated in **Table 7**, in which the "per 100 g protein" or "per L of soymilk/bovine milk" data of Poore & Nemecek (2018) are adjusted for protein quality by dividing by published DIAAS values, where suitable DIAAS data are available.

DIAAS values for the protein-rich foods in this study vary between 0.434 for roasted peanuts (Rutherfurd et al. 2015) and 1.32 for bovine milk (Mathai et al. 2017), which means that the correction for protein quality results in a 130% increase in the impact of peanuts and a 24% decrease for milk; i.e., the footprint ratio of peanut to bovine milk changes by more than 200%! Other comparisons change less dramatically with an adjustment for protein quality, e.g., a 30% relative change for the comparison between soy milk (DIAAS 1.015) and bovine milk.

Table 7 Estimated greenhouse gas emissions in kg CO_2eq per liter (milk and soy milk) or per 100 g protein (all others) for production of protein-rich foods (Poore & Nemecek 2018)[a]

Product	Greenhouse gas emissions (kg CO_2 equivalent/100 g protein)						DIAAS	Adjustment difference
	Original			DIAAS-adjusted				
	10%[b]	Median	90%[c]	10%	Median	90%		
Bovine meat (dairy herd)	9.09	17.29	25.79	8.14	15.50	23.11	1.116[d]	−10%
Bovine meat (beef herd)	20.25	30.27	105.24	18.14	27.12	94.30	1.116[d]	−10%
Cheese	4.95	8.44	17.81	3.51	5.99	12.63	1.41[e]	−29%
Tofu	1.00	1.61	3.47	0.99	1.59	3.42	1.015[f]	−1%
Nuts	−2.24	−0.81	2.35	−5.15	−1.88	5.42	0.434[g]	+130%
Peas	0.25	0.36	0.75	0.35	0.49	1.03	0.73[h]	+37%
Groundnuts	0.62	1.26	2.22	1.43	2.90	5.11	0.434[g]	+130%
Other pulses	0.46	0.65	1.75	0.78	1.10	2.98	0.588[i]	+70%
Soymilk[k]	0.58	0.91	1.47	0.57	0.90	1.45	1.015[f]	−1%
Milk[k]	1.70	2.65	4.83	1.29	2.01	3.66	1.32[j]	−24%

[a]Emissions data are shown as originally reported and after adjustment for the nutritional quality of protein sources, as measured by published digestible indispensable amino acid scores (DIAAS).
[b]Tenth percentile.
[c]Ninetieth percentile.
[d]Value for beef, as calculated using true ileal digestibility in pigs and reference requirements for six-month-old to three-year-old children (Ertl et al. 2016).
[e]DIAAS for milk protein concentrate using digestibility measured in pigs and reference requirements for children 3 years and above (Mathai et al. 2017).
[f]Average between DIAAS values of soy protein isolate and soy flour (Mathai et al. 2017).
[g]DIAAS for roasted peanuts (Rutherfurd et al. 2015), as measured in growing male rates and calculated with reference to requirements for 6-month- to 3-year-old children.
[h]Value for cooked peas (Rutherfurd et al. 2015).
[i]Value for cooked kidney beans (Rutherfurd et al. 2015).
[j]Average between DIAAS values for milk protein concentrate and skim milk powder (Mathai et al. 2017).
[k]Units are kg CO_2 equivalent/L, standardized at 3.3% protein.

Adjusting footprint data for DIAAS does not change the overall conclusion that producing beef has a higher environmental impact than for other protein-rich foods. However, White & Hall (2017) pointed out certain resource efficiencies specific to animal agriculture; e.g., animals can process human-inedible agricultural by-products into edible materials, and their manure reduces the need for synthetic fertilizer. Animals can make use of pasture and grazing lands that are untillable or "marginal" and therefore not suitable for crop production, and this lessens the incentive to convert forest to farmland. The capability of marginal land to support edible crops was quantitatively modeled by van Zanten et al. (2016), who proposed a soil-specific "land use ratio" to express the efficiency of plant- versus animal-based cultivation. The higher micronutrient content and bioavailability in animal-based foods (Ertl et al. 2016, White & Hall 2017) somewhat counteract higher production inputs.

Poore & Nemecek (2018) noted that environmental impact data were often skewed by a minority of high-impact producers, which highlighted an opportunity for targeted mitigation of impact by modifying farming practices. Reduction of environmental impact does not necessarily compromise profitability; in fact, O'Brien et al. (2015) showed that the Irish dairy farms with the lowest carbon footprint were those with the highest economic performance and lowest concentrate feeding. In Canadian dairy farming systems, on-farm emissions account for approximately 90% of total emissions, and the off-farm component varies substantially between dairy product types (Vergé et al. 2013).

EMERGING PROTEIN SOURCES

Many new food protein sources are discussed in the literature, but not all of them are commercializable. Food ingredient wholesalers typically will not stock a new protein ingredient until it fulfills certain conditions:

- Available in kiloton quantities at reasonable cost
- Minimal batch-to-batch or seasonal variation
- Chemically and microbially stable for at least 12 months at ambient temperature
- Permitted for food uses in major jurisdictions

Cultured meat, insect-derived proteins, and algal proteins are discussed below, and promising new plant-derived proteins are highlighted in **Table 8**.

Cultured Meat

> We could cut our beefsteak from a tissue culture of muscle with no nervous system to make it waste food in doing work, and a supply of hormones to make it grow as fast as that of an embryo calf.
>
> —J.B.S. Haldane (1927)

The prospect of producing meat products without animals was conceived more than 90 years ago in an essay by J.B.S. Haldane (1927). Cultured meat was demonstrated in principle in 2013 when a team from Maastricht University produced a burger patty comprising bovine cells grown in a laboratory (Jha 2013).

Producing cultured meat involves isolating skeletal muscle stem cells (myosatellite cells) from an animal, inducing cells to proliferate and differentiate in culture medium, and engineering tissue structures (Post 2012), as illustrated in **Figure 8**. Cultured meat should be distinguished from meat analogs (also known as meat mimics, meat alternatives, imitation meat, or mock meat), which have meat-like texture, color, and flavor but do not contain muscle tissue.

Tissue engineering is one of the greatest challenges of cultured meat production. This requires three factors (Langelaan et al. 2010):

Table 8 Overview of emerging plant protein sources

Source	Comments	References
Pea	Good emulsification and foaming, poor water binding	Geerts et al. 2017 Lam et al. 2018 Peng et al. 2016 Stone et al. 2015
Beans	Similar functionality to pea protein, functionality depends on extraction, high-pressure homogenizing improves functionality	Multari et al. 2015 Shevkani et al. 2015 Yang et al. 2018
Lupin	High thermal stability, low viscosity	Berghout et al. 2015 Coorey et al. 2011
Potato	Good heat-gelation, emulsification	Creusot et al. 2011 Delahaije et al. 2014
Green leaves	Low solubility, major soluble protein (rubisco) has good heat gelation and forms brittle gels	Martin et al. 2014 Tamayo Tenorio et al. 2016

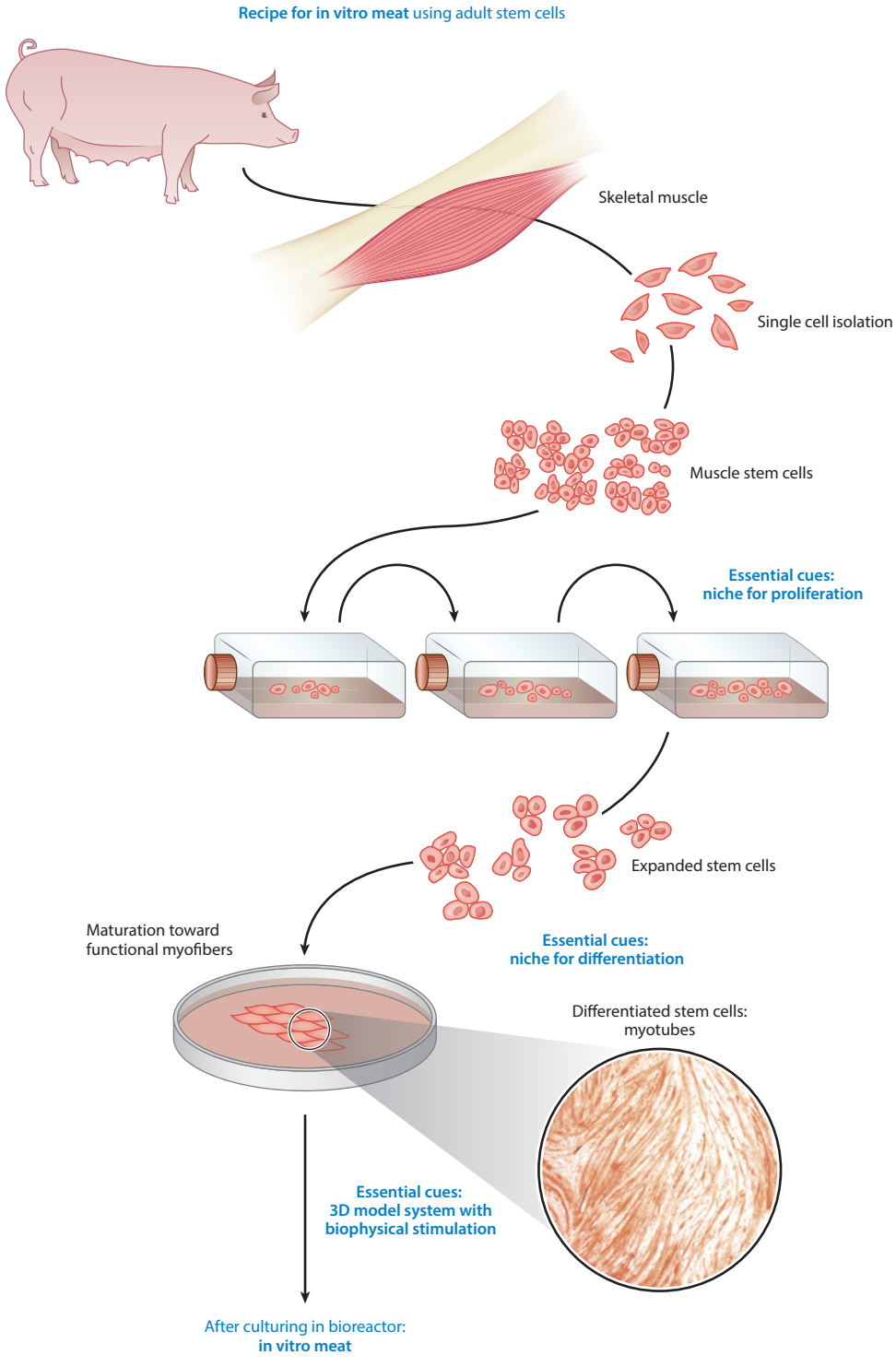

Figure 8

Schematic depiction of cultured meat production. Adapted with permission from Langelaan et al. (2010).

- A cell source that can proliferate indefinitely. This is discussed further in Kadim et al. (2015)
- A supporting solid matrix or scaffold that allows for muscle growth while maintaining oxygen and nutrient levels via passive diffusion in the absence of a vascular system
- Biophysical, biochemical, and electrical stimulation, without which muscle cells do not mature properly

It may be possible to overcome some of these challenges by coculturing myoblasts (muscle cells) with fibroblasts to produce an extracellular matrix (Brady et al. 2008) and vascular cells (Jain et al. 2005) or bioprinted blood vessels (Skardal et al. 2010) to transport nutrients and waste metabolites. Fat is an important contributor to the flavor and juiciness of meat, and adding adipocytes to the coculture (Hausman & Poulos 2005) or when forming finished products (Post 2018) may improve the sensory properties of cultured meat.

The scale-up of muscle cell culture to large-scale production poses particular challenges in the differentiation and maturation phases, where solid substrate materials must enable anchoring and contraction of muscle cells while facilitating nutrient supply and waste metabolite removal. The proof-of-principle cultured burger patty was created by growing a multitude of cell sheets only a few hundred micrometers thick; the thickness of sheets is limited by poor nutrient diffusion into and out of cells at greater thicknesses (Kadim et al. 2015). More efficient cell production configurations include either growth on microcarrier beads, cultivation as cell aggregates, or immobilization of cells in packed-bed reactors (Moritz et al. 2015). Other possibilities include electrospun fibers, micropatterned surfaces, or 3D-printed scaffolds, potentially composed of edible materials (Datar & Betti 2010).

The resource-intensity of cultured meat production is difficult to gauge because large-scale production has not yet been realized at the time of writing. A life-cycle analysis of cultured meat production was attempted in 2011 (Tuomisto & Teixeira De Mattos 2011), but it was by necessity so speculative and simplified as to be of limited value. A more sophisticated and realistic analysis was published four years later (Mattick et al. 2015), and this suggested that producing cultured meat would require more energy than producing comparable quantities of conventional meat. A sensitivity analysis indicated the potential for dramatically higher energy use in cultured meat production than for the beef, pork, or poultry comparator studies (only one on each meat), and indicated that land use would be dramatically lower for cultured meat production. At present, there is insufficient literature to draw robust conclusions about the resource intensity or environmental impacts of industrial cultured meat production.

Cultured meat product research is currently attracting vast amounts of public interest and venture capital (Dance 2017) and producing very little scientific literature, probably because it occurs mainly in a competitive industry context. The technology is advancing rapidly and costs are coming down by orders of magnitude (Heffernan 2017). The proof-of-principle burger patty had a texture and flavor similar to that of minced beef. However, tissue engineering challenges mean that cultured meat products replicating the appearance, aroma, mouthfeel, and flavor of whole-muscle meat cuts are still a long way from reality. The regulatory status and labeling requirements for cultured meat are currently under debate (Servick 2018); the safety and nutritional qualities of cultured meat properties have yet to be reported.

Insect Proteins

Insects have been eaten traditionally for thousands of years (Ramos-Elorduy 2009), but the industrialization of insect rearing and processing for food is relatively new. A wide variety of insects can potentially be consumed for food, e.g., crickets, locusts, grasshoppers, caterpillars, beetles,

Table 9 Protein, fat, and energy content of selected insects and comparator food materials[a]

Insect or food material	Protein (% dry matter)	Fat (% dry matter)	Energy (kcal/100 g)
Coleoptera (adult beetles, larvae)	40.69	33.4	490.3
Rhynchophorus phoenicis (palm weevil larvae)	32.86	36.86	478.87
Tenebrio molitor (mealworm larvae)	48.35	38.51	557.12
Diptera (flies)	49.48	22.75	409.78
Hemiptera (true bugs)	48.33	30.26	478.99
Hymenoptera (ants, bees)	46.47	25.09	484.45
Oecophylla smaragdina (weaver ant)	53.46	13.46	NA
Isoptera (termites)	35.34	32.74	NA
Lepidoptera (butterflies, moths)	45.38	27.66	508.89
Bombyx mori (silkworm larvae)	61.8	8.81	389.6
Cirina forda (shea caterpillar)	47.48	11.5	359
Galleria mellonella (waxworm larvae)	38.01	56.65	650.13
Samia cynthia ricini (ailanthus silkworm pupae)	54.7	25.6	463.63
Odonata (dragonflies, damselflies)	55.23	19.83	431.33
Orthoptera (crickets, grasshoppers, locusts)	61.23	13.41	426.25
Acheta domesticus (house cricket adult)	65.04	22.96	455.19
Schistocerca sp.	61.05	17	427
Sphenarium purpurascens (chapulin adult)	61.33	11.7	404.22
Ruspolia differens (brown longhorn grasshopper)	44.3	46.2	NA
Skim milk powder[b]	37.3	0.80	373.8
Whey protein isolate[c]	92.0–96.1	0.4–1.0	NA
Soy protein isolate[d]	92.9	3.57	353
Raw beef[e]	81.2	14.1	454.9

[a]Adapted with permission from Dobermann et al. (2017), with additional data from sources indicated in footnotes below.
[b]USDA Food Composition database entry 01091: milk, dry, nonfat, regular, without added vitamin A and vitamin D.
[c]Foegeding et al. (2002).
[d]USDA Food Composition database entry 16122: soy protein isolate.
[e]USDA Food Composition database entry 23427: New Zealand manufacturing beef, raw.
Abbreviation: NA, not available.

ants, and fly larvae (Schlüter et al. 2017). Each insect type, developmental stage, and cultivation/processing scenario should be considered on a case-by-case basis because of wide variation in chemical composition and case-specific hazards.

Insects are a potentially rich source of protein and lipids, as shown in **Table 9**, as well as micronutrients and minerals (EFSA 2015, Ramos-Elorduy et al. 1997, Schlüter et al. 2017). They may also be a source of bioactive peptides, polyunsaturated lipids, sterols, and polysaccharides (Sun-Waterhouse et al. 2016). Currently, there is little known about the bioavailability of insect-derived nutrients for humans. In vitro protein digestibilities of insect proteins in the 77%–98% range have been reported (Ramos-Elorduy et al. 1997), and rat fecal digestibility of honeybee proteins is relatively high (Ozimek et al. 1985). Lysine and tryptophan are often the limiting indispensable amino acids (EFSA 2015).

A number of insect-derived food powders are available, but for the most part they contain dried, ground whole insect, and little is known about the extraction and purification of insect proteins for food ingredients. Ndiritu et al. (2017) extracted cricket protein by hexane or aqueous extraction and found that hexane extraction gave higher protein yield and a lighter-colored product, but aqueous-extracted cricket protein had better emulsifying and foaming functionality.

Aqueous extracts from freeze-dried powders of five insects were produced and characterized by Yi et al. (2013). The protein profile and foaming and gelling functionality of extracted fractions were tested. The water-soluble protein was 23% of total protein at best and foaming functionality was poor, but the aqueous extract from the lesser mealworm (*Alphitobius diaperinus*) and Dubia cockroach (*Blaptica dubia*) formed strong gels at 15% w/v.

In the work of Mariod & Fadul (2015), melon bug (*Coridius viduatus*) and sorghum bug (*Agonoscelis versicoloratus*) were extracted with hot water, mild acid, or cold water, and extracts were tested for their potential to replace gelatin in ice cream. In sensory testing, experimental insect ice creams received significantly lower taste and texture preference scores than a commercial gelatin ice cream (Mariod & Fadul 2015), but this is perhaps a reflection that commercial gelatin ingredients are the result of hundreds of years of process refinement.

Insect exoskeletons comprise primarily chitin, a polymer of *N*-acetyl-D-glucosamine. In one case, alkali extraction of honeybees removed chitin and improved the fecal digestibility of protein in rats (Ozimek et al. 1985), although causality was not proven. The corollary of this result is that chitin may inhibit protein digestion. However, chitinase activity has been reported in human gastric juices (Muzzarelli et al. 2012) and gastrointestinal microbiota (Dobermann et al. 2017), and chitin-based food ingredients have been approved for use in the EU (EFSA 2010).

A prerequisite for considering an insect species and its developmental stage as a human food source is that it produces low levels of endogenous toxins or antinutrients, and this has been verified in several cases (Dobermann et al. 2017). The allergenic potential of insects is cause for concern. Cross-reactive allergies to insects occur in people with allergies to crustaceans and dust mites (Ribeiro et al. 2018).

Insects can accumulate contaminants from their feed or housing materials, especially when fed on organic waste materials. Given that evisceration and surface decontamination of farmed insects are problematic, surface contaminants and the gut contents at the time of slaughter will carry through to the processed product or ingredient. The digesta can contribute substantially to the nutrient composition, toxic and allergenic potential, and microbial load of insect-derived foods (Dobermann et al. 2017).

The regulatory status of insect-based foods is summarized by jurisdiction in **Table 10**. Because of a lack of knowledge about the safety of insect-derived foods (EFSA 2015), whole insects and their parts are considered a "novel food" under EU Regulation 2015/2283 and carry similar regulatory status in North America, but certain exceptions are permitted in the Netherlands and Belgium (Dobermann et al. 2017).

Algal Proteins

Algae have been consumed as food for hundreds of years (Ścieszka & Klewicka 2018), but the extraction and purification of algal protein are relatively new. Unicellular microalgae such as *Arthrospira platensis* (spirulina in common parlance because of earlier classification in the genus *Spirulina*) and *Chlorella* species have been given Generally Regarded as Safe (GRAS) status and are produced commercially in bioreactors or open ponds. They contain 21–70% and 51–58% protein, respectively, as a proportion of dry weight (dw) (Bleakley & Hayes 2017, Teuling et al. 2017).

The macroalgae are multicellular marine or freshwater plants (seaweeds), some of which are farmed commercially and used as a source of polysaccharides and animal feed, or eaten as vegetables. The protein content can reach 25–45% dw for several of the red seaweeds, whereas brown seaweeds typically have <15% dw protein (Chronakis & Madsen 2011).

Table 10 Regulatory status of insects as food and feed in 2017 (Lähteenmäki-Uutela et al. 2017)

Countries	Insect as food market situation	Laws on insects as food	Laws on insects as feed
European Union	Some countries have some insect foods on the market, others none	Novel Food Regulation applies, 2018 rules acknowledge use in third countries	Animal-based material banned as feed, ban lifted on feed for aquaculture
United States	Some insect food products on the market	No novel food regulation: additive approval or GRAS needed	Normal feed rules apply: additive approval or GRAS needed for insects
Canada	Some insect food products on the market	Insects used traditionally anywhere in the world are not novel	Feed raw material needs authorization, one black soldier fly product authorized for poultry
Mexico	Several insect food products on the market, mainly gathered insects	Organic insects are regulated, GMO is regulated, no novel food regulation	Feed materials generally do not require registration
Australia	Some insect food products on the market	Traditional foods and non-novel foods can be marketed	Feed materials generally do not require registration
China	Several insect food products on the market	Insects can be used in health foods, novel food regulation applies to normal foods	New feed materials require authorization

Abbreviations: GMO, genetically modified organism; GRAS, generally regarded as safe.

Algal protein isolates and concentrates have been produced from several microalgae. Extraction typically starts with bead milling or ultrasound processing to disrupt cell walls (Tamayo Tenorio et al. 2018, Yucetepe et al. 2018), and protein may be extracted in alkali (Cavonius et al. 2015, Pereira et al. 2018) or by centrifugation, dialysis, and anion exchange (Teuling et al. 2017). Further purification can be achieved by acid precipitation (Cavonius et al. 2015, Pereira et al. 2018, Teuling et al. 2017). The major protein classes in algae are rubisco (ribulose-1,5-bisophosphate carboxylase/oxygenase) and chlorophyll-containing light-harvesting complexes, both of which are multimeric (Teuling et al. 2017).

Algal proteins have poor solubility at pH 3–5, especially at high ionic strength, and this phenomenon is common to protein extracts from unrelated species of microalgae (Cavonius et al. 2015, Pereira et al. 2018, Schwenzfeier et al. 2011, Teuling et al. 2017). However, at neutral pH, algal proteins show promising functionality in foaming (Pereira et al. 2018, Schwenzfeier et al. 2013b) and emulsification (Schwenzfeier et al. 2013a).

Calculated nitrogen-to-protein conversion factors (see sidebar titled Protein Quantification) for algal proteins range from 3.88 to 6.35 depending on the algal species and protein extraction protocol (Teuling et al. 2017, Tibbetts et al. 2016, Wells et al. 2017). The major factors driving this wide variation appear to be different amino acid profiles and the presence of varying amounts of non-protein nitrogen (Teuling et al. 2017, Tibbetts et al. 2016).

True ileal digestibility data for algal proteins are not available at present. Methionine, cysteine, lysine, and tryptophan have been reported as the limiting amino acids in algal protein (Bleakley & Hayes 2017, Wong & Cheung 2001), and in vitro protein digestibilities of 78.4%–86.7% (Tibbetts et al. 2016) and 85.7%–88.9% (Wong & Cheung 2001) have been measured for various seaweeds. In vivo studies suggest that soluble polysaccharides and oxidized polyphenols present at high levels in algae may inhibit protein digestion (Bleakley & Hayes 2017).

CONCLUSIONS AND FUTURE PROSPECTS

Many new food proteins are becoming available, and they present unknown opportunities and risks. Risks relating to allergens, contaminants, and toxins that may be associated with new protein sources deserve careful consideration. These new proteins may have the potential to alleviate malnutrition, mitigate the environmental impact of producing food protein, reduce manufacturing costs, or improve the quality of formulated foods. Blending proteins from different sources may produce functional and/or nutritional synergies, and this potential is largely unexplored. In the face of societal and technological change, the fundamentals of protein chemistry, biophysics, and human nutrition remain the best platform for responsible food innovation.

SUMMARY POINTS

1. Food proteins supply essential amino acids and play technological roles in foods.
2. Nutritional and technological properties depend on protein source, extraction and purification, modification during food manufacture, and interactions with other food proteins.
3. Nutritional quality includes both the content of essential amino acids and their true ileal digestibility.
4. Environmental sustainability comparisons should include protein quality measures.
5. Cultured meat products with mince-like texture can be produced from reactor-grown animal cells, but tissue engineering challenges currently preclude convincing substitutes for whole-muscle meat.
6. Many insects are rich in protein, and with sufficient attention to hygiene and toxicity considerations, they could become a mainstream source of food protein.
7. Algae are easily cultivated and often rich in protein, but the heterogeneity of algal protein and its low solubility at acidic pH pose challenges for food functionality.

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