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

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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 a wide range of structural and functional roles in the body; without protein intake we would die. Protein-based ingredients fulfil many different technological roles in formulated foods, and contribute to texture, colour, flavour and other properties. This review will examine 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 will describe how this complexity is manifested in the properties of high-protein food ingredients, and how it can be understood and utilised to formulate safe and delicious foods that also deliver high-quality protein nutrition.

Table 1. Protein intake data by life stage, compiled from 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 to 6 mo	1.12*	9.1**	6
6-12 mo	1.0	11.0	9
1-3	0.87	13	12
4-8	0.76	19	20
Men			
9-13	0.76	34	36
14-18	0.73	52	61
>18	0.66	56	70
Women			
9-13	0.76	34	37
14-18	0.71	46	54
>18	0.66	46	57
Pregnancy	0.88	71	-
Lactation	1.05	71	-

a grams of protein per kilogram of body weight per day
b grams per day, based on reference body weights
* from (World Health Organization 2007)
** Adequate Intake: mean intake for healthy breast-fed infants

A large research effort has gone into establishing how much protein we need to eat to remain healthy, and these guidelines are summarised in Table 1. Estimated Average Requirements (EAR) 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 percent) healthy individuals in a group (Institute of Medicine 2005). There is some debate about RDAs for adults over the age of 65, and indications that for this group an intake in 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 a 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 from around 10,000 B.C..

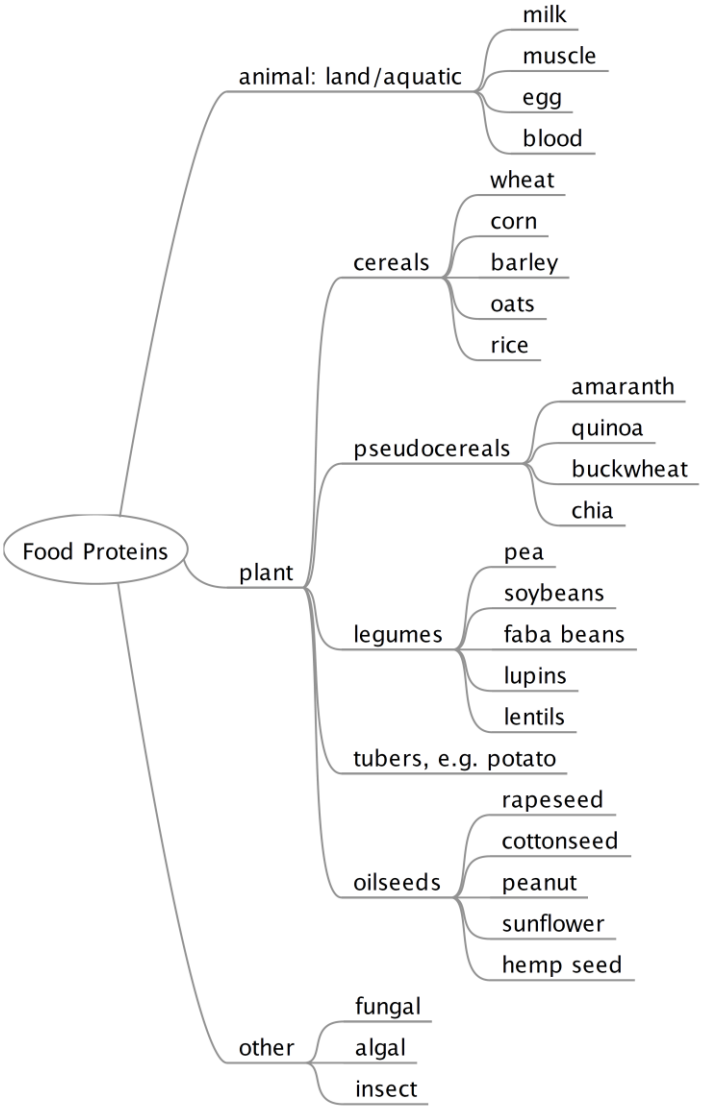

Figure 1. Major sources of food protein.

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 19th 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. 0.1M NaCl
- Prolamins: insoluble in water, soluble in 70-80% aqueous ethanol and heat-resistant
- Glutelins: insoluble in water, soluble in dilute alkali, e.g. 0.1M 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** summarises the fraction in various plant proteins. It can be seen that legumes contain predominantly albumins and globulins, whereas cereal protein is dominated by prolamins and glutelins, which explains why non-dairy milk substitutes made from cereals (and nuts) are very low in protein (Vanga and Raghavan 2018).

Table 2. Summary of protein content by class in various plant-derived foods, from Day (2013).

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		90%		
Pea	15-25%	50-60%		
Chickpea	8-12%	53-60%	3-7%	19-25%
Lupin	25%	75%		
Canola	20%	60%	2-5%	15-20%

Albumins and globulins can be gravimetrically fractionated by ultracentrifugation-derived Svedberg sedimentation coefficients, expressed in Svedberg units, S. The albumins are predominantly 2S proteins whereas globulins occur in 7-8S and 11-12S groups (Häkkinen, et al. 2018, Shewry and 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 and 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 and 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 solutions.

A knowledge of these fractions is particularly important to the manufacture of surimi (Park and Lin 2005). The removal of sarcoplasmic proteins from fish meat during washing, and the retention of myofibrillar proteins gives the highest quality and yield. An ionic strength of 0.01 – 0.1 and pH 5.5 minimises the solubility of myofibrillar proteins and optimises their separation from sarcoplasmic proteins (Stefansson and 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 solubilised by acid- alkali- and/or heat-induced denaturation and hydrolysis, as in the conversion of insoluble collagen into soluble gelatin (Haug and Draget 2011). Heat-sensitive

proteins such as whey protein can be enzymatically hydrolysed 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 *in toto*, e.g. mycoprotein from fungal sources, algal proteins, and insect-derived proteins. This heterogeneity is particularly evident in polyacrylamide gel electrophoretograms (Yi, et al. 2013).

Protein Purification

Mammalian milk proteins are readily fractionated with acid or rennet (Figure 2). Rennet is an enzyme that hydrolyses the kappa caseins that form a 'hairy layer' on the surface of native casein micelles, and thereby removes steric stabilising forces, leading to self-association and precipitation of micelles. Acid destabilises casein micelles by neutralising the charges on surface kappa casein molecules so that they collapse onto the surface of micelles, which are thus destabilised. These phenomena are discussed by Dalgleish (2014).

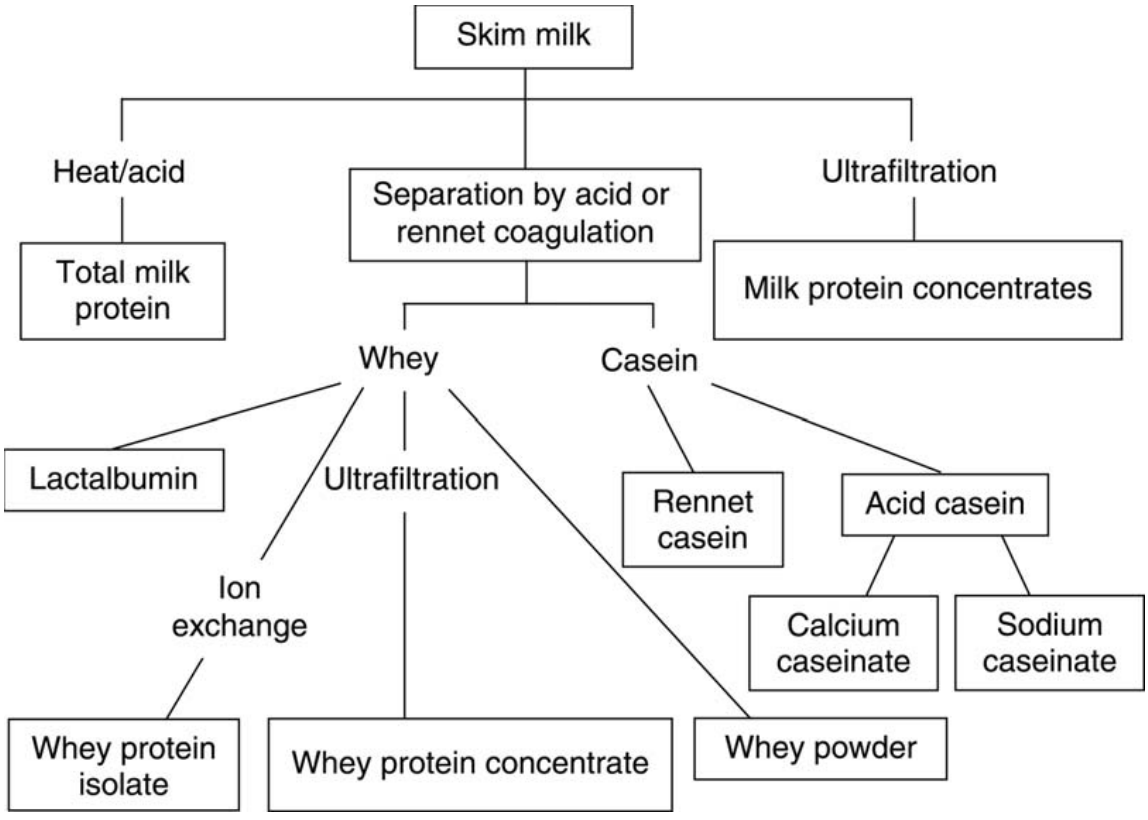

Figure 2. Industrial milk protein fractionation scheme, from Singh (2011).

Native whey proteins are resistant to the action of rennet; at high enough concentration they will 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). 'Lactalbumin' in Figure 2 refers to an insoluble powder produced by heat-precipitating whey proteins (Matthews 2014); the protein α-lactalbumin is the second most abundant component of bovine whey protein, after β-lactoglobulin.

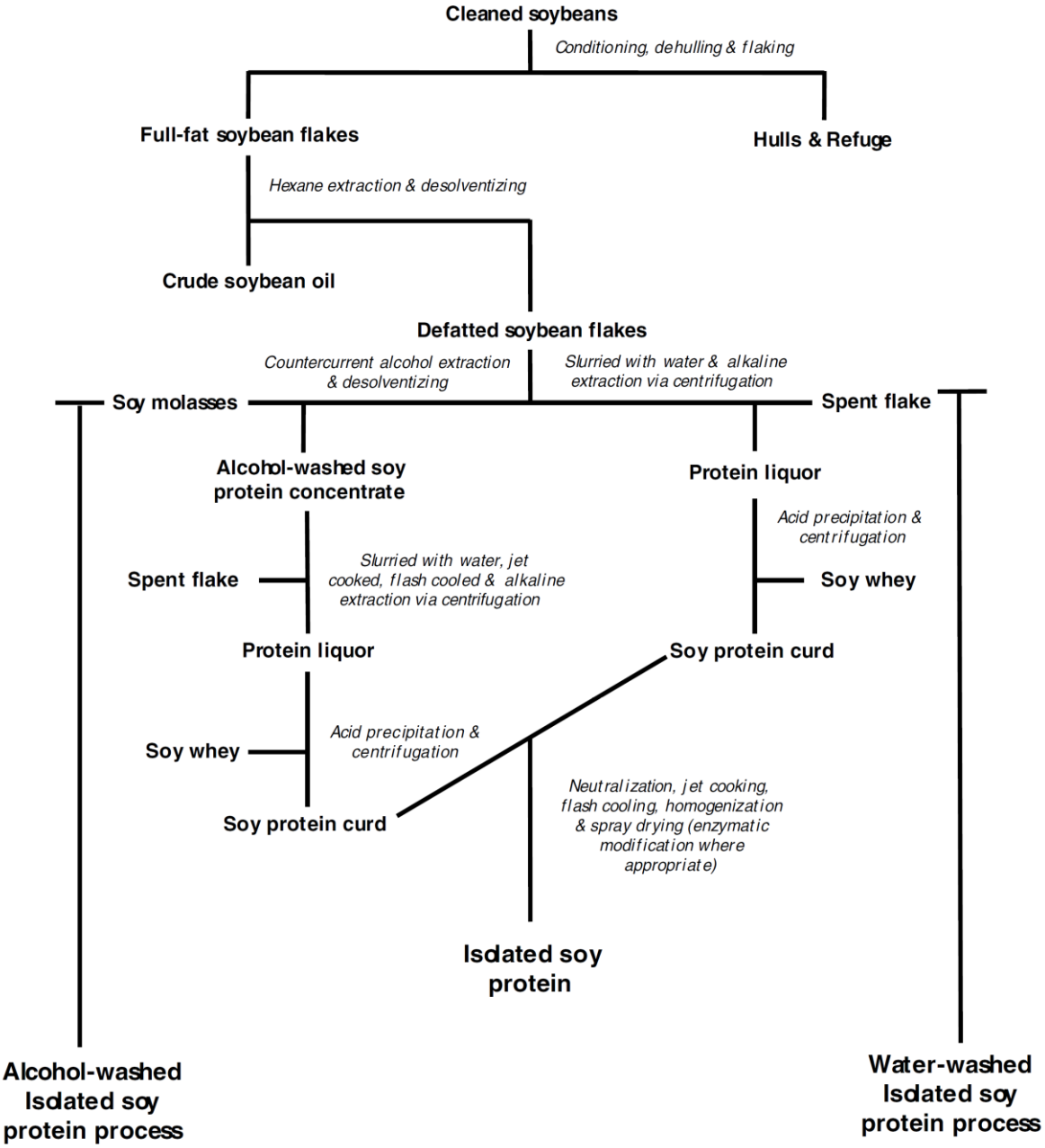

Figure 3. Isolated soy protein extraction and purification processes, from Egbert (2004).

Extraction and purification of plant protein is more involved, which reflects the fact that plant proteins are often sequestered as insoluble, inert bodies within seeds. The extraction and purification of soy proteins is depicted in Figure 3. Extraction of defatted soy flakes with alkaline or alcoholic solutions produces soy protein concentrate, which has low solubility unless further processed by heating, homogenising 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 protein content in the region of 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 and 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), mechanical support and movement (muscle proteins), carrying oxygen or vitamins (haemoglobin, lipocalins) or 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 involves quite severe heat, shear, and/or solvent extraction processes, which inevitably leads to some degree of denaturation, crosslinking and even hydrolysis. The kinetics of heat-induced protein denaturation depends on the heating temperature and the ionic environment, particularly pH and ionic strength (Loveday 2016).

Table 3. Functionalities of proteins in food.

Functionality	**Example**
Crosslinking	enzyme, e.g. transglutaminase heat-induced gelation acid gelation polyvalent ions cold-set gels
Solubility	heat stability in beverages
Emulsification	conventional emulsions Pickering emulsions
Flavour/aroma	meaty/roasted notes (cysteine)
Colour	Maillard (nonenzymatic) browning
Antimicrobial	lysozyme, lactoferrin
Texturisation	meat analogues
Foaming	foaming capacity foam stability
Water holding	yoghurt, processed meat

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).

Table 4. Functionalities of pea protein isolates produced by different extraction methods from the CDC Striker cultivar, adapted from Stone, et al. (2015).

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

a: alkali extraction-isoelectric precipitation
b: micellar precipitation
c: salt extraction

At sufficiently high protein concentration, heat-denatured proteins will aggregate, particularly via hydrophobic interactions, hydrogen bonds and disulphide 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 leads to filamentous or fine-stranded aggregates with a 'string of beads' morphology. Aggregation is more random at moderate or high ionic strengths (>~50 mM NaCl or >~10 mM $CaCl_2$ (Bryant and McClements 1998)) or at pH approaching the pI, leading to particulate aggregates (Ako, et al. 2009, Doi 1993).

Filamentous aggregates will 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 and McClements 1998). For that reason, gelling temperature strongly influences gel properties (Bryant and McClements 1998). These fine-stranded or 'homogeneous' cold-set gels are typically transparent (Ako, et al. 2009).

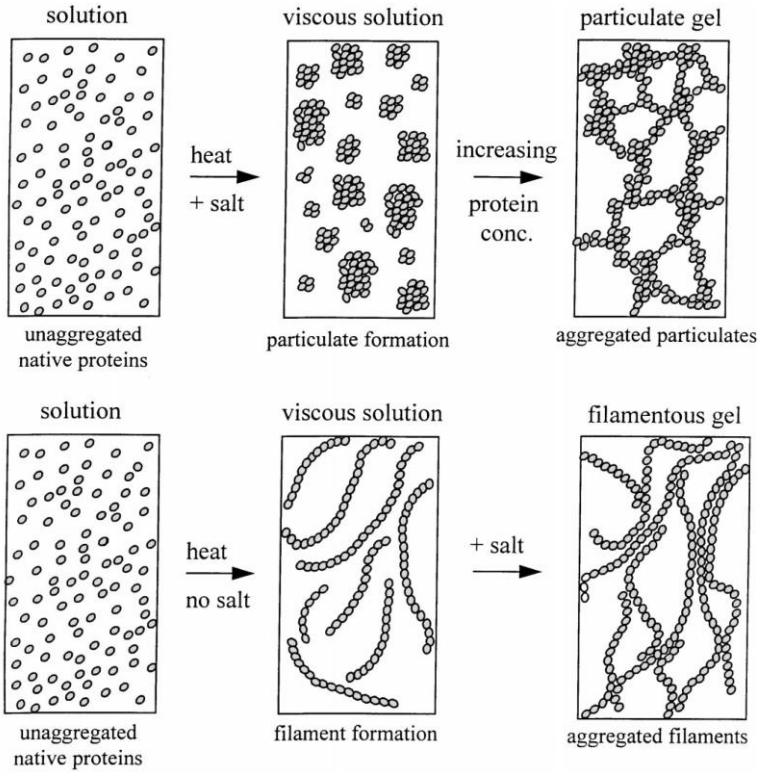

Figure 4. Development of particulate and fine-stranded (filamentous) protein gel structures, from Bryant and McClements (1998).

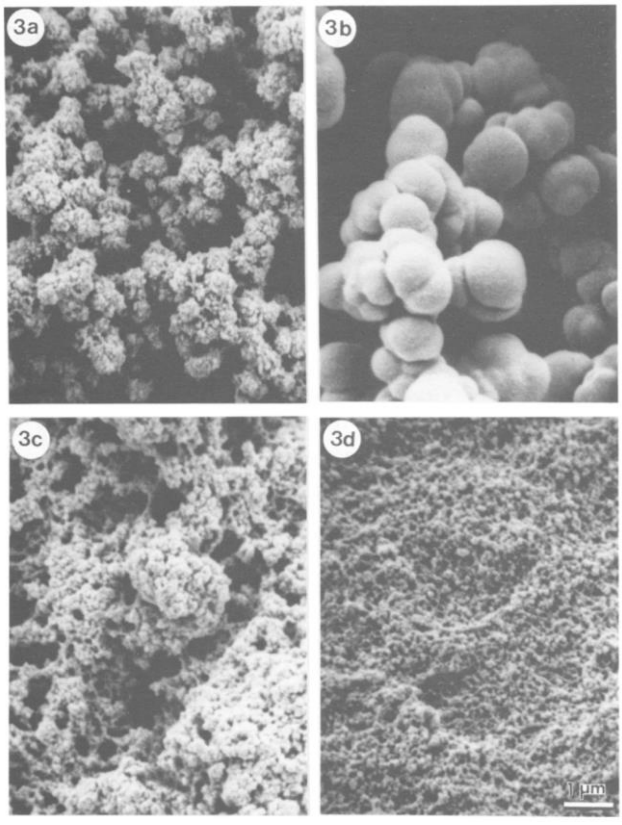

Figure 5. Scanning electron micrographs of heat-set β-lactoglobulin gels formed at different pH - A: pH 6, B: pH 5.5, C: pH 4.5, D: pH 4. From Langton and Hermansson (1992).

Particulate or 'heterogeneous' gels are more turbid and typically have lower water holding capacity. The transition between fine-stranded and particulate aggregation is very sensitive to pH, as show in Figure 5 (Langton and Hermansson 1992), and this reflects micro-phase separation phenomena (Ako, et al. 2009). The rubberiness (fracture strain) of heat-set whey protein gels depends on the degree of disulphide 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 and Foegeding 1990).

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

Protein Digestion

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

Liquid beverages pass through the oral phase almost 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 and Chen 2017). Salivary mucins can interact with protein-stabilised 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 and Macierzanka 2010, Sarkar, et al. 2009).

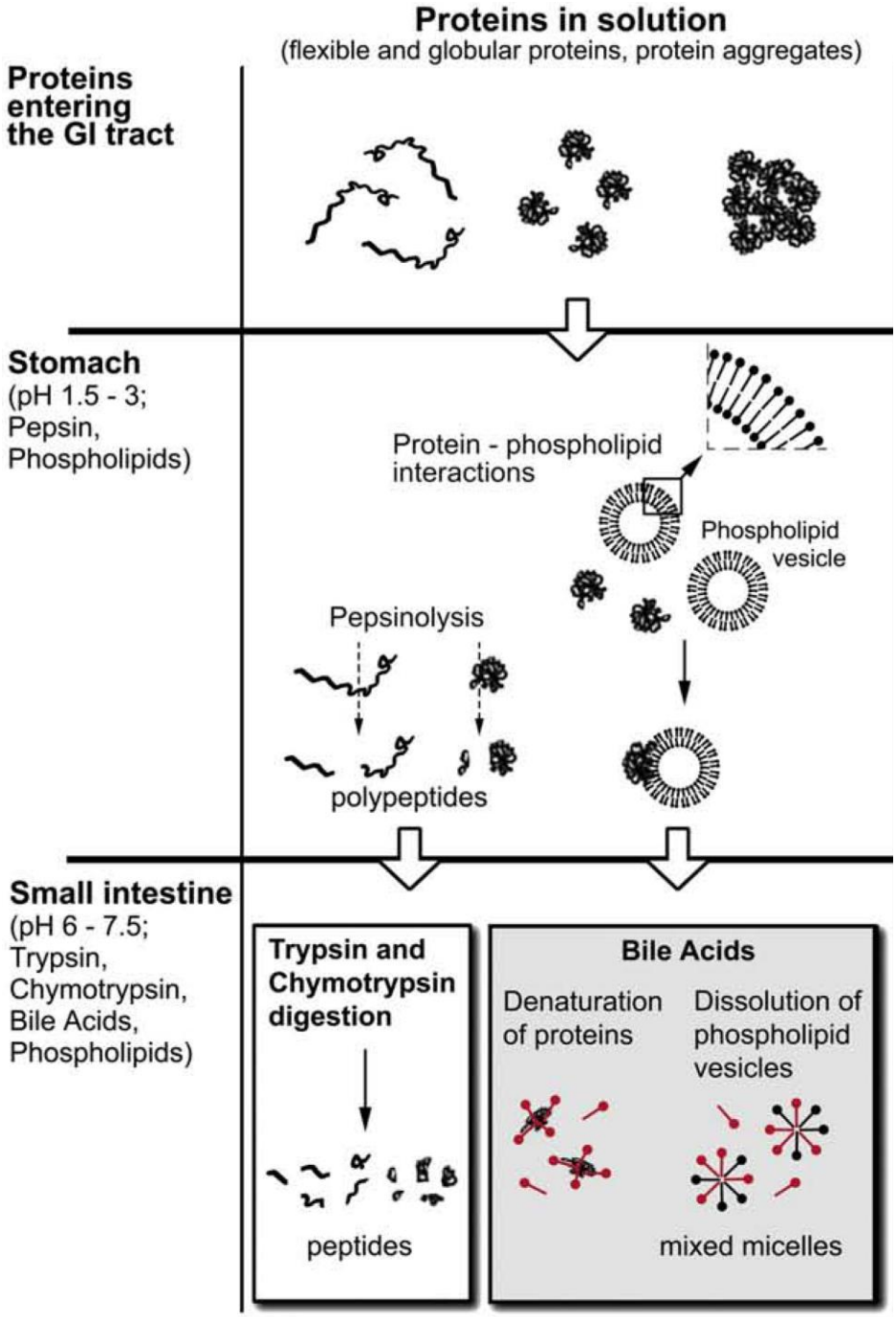

Figure 6. schematic illustration of protein digestion processes at various stages of the gastrointestinal tract, adapted from Mackie and Macierzanka (2010).

The major chemical changes in 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-resistant and pepsin-resistant in its native form, but the heat-denatured form is rapidly hydrolysed by pepsin (Peram, et al. 2013).

The caseins appear to be unique in their ability to form a coagulum in the stomach when in the micellar form that prevails in milk. Casein coagulation under gastric conditions is driven by the cleavage of kappa-caseins by pepsin at relatively high pH (Ye, et al. 2016), which removes steric stabilisation. Even partially micellar forms of casein such as sodium caseinate and casein complexes with whey or fat globules in heated/homogenised milk will coagulate somewhat under gastric conditions due to the loss of electrostatic repulsion at acid pH (Ye, et al. 2016, Ye, et al. 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 alkaline pancreatic secretions that largely neutralise the pH, and hydrolytic enzymes trypsin and chymotrypsin. Intestinal proteolysis is remarkably effective, and most proteins are reduced to di- or tri-peptides. 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 proteins are partially digestion-resistant and can be absorbed. Even bioactive peptides that are not absorbed can modulate gastrointestinal function 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 Jr, 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 (see side bar)
- Proportions of different amino acids
- Digestibility

Intriguingly, the nutritional benefit of consuming protein exceeds that of consuming the constituent essential amino acids in corresponding quantities (Katsanos, et al. 2008).

[SIDEBAR] Protein quantification

Protein content can be measured with a range of different assays (see table). 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 assays, and the required level of accuracy (Moore, et al. 2010). Certain solvents, detergents, chelators and reducing agents can interfere with dye-binding methods (Noble and Bailey 2009). Several assays are sensitive to amino acid sequence, particularly those measuring aromatic amino acids (A_{280}), and protein standards should be selected with care (Moore, et al. 2010, Noble and Bailey 2009).

PRINCIPLE	EXAMPLES	REFERENCE
Spectroscopy	Absorbance at 280 nm (Tyr, Trp)	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-Ciocalteau 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	

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 (International Dairy Federation 2016). Protein measurement standards are maintained by the Codex Alimentarius Commission Committee on Methods of Analysis and Sampling (Standard CXS 234-1999: Recommended Methods of Analysis and Sampling).

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 soyabean 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 derivatives of lysine modification are often acid-labile, which means that available lysine is measured differently to total lysine (Rutherfurd and 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 and Rutherfurd 2012). Protein digestibility ranges for various food materials are summarised in Figure 7, and digestibility data for ten food ingredients and eleven foods commonly consumed in India can be found in Rutherfurd, et al. (2012).

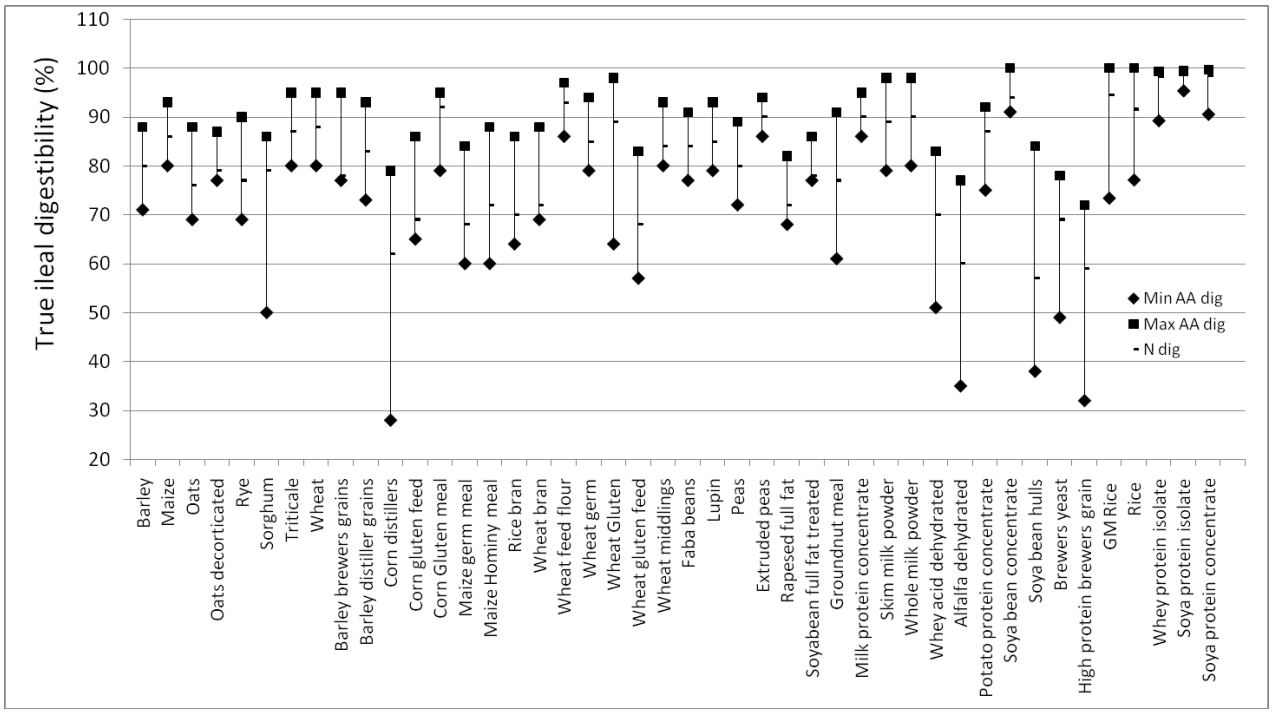

Figure 7. Maximum true ileal amino acid digestibility (■), minimum true ileal amino acid digestibility (♦), and true ileal nitrogen digestibility (-) of amino acids in protein-rich foods and feeds, from Moughan, et al. (2012).

Of the 20 amino acids utilised in human metabolism, 9 are considered essential or indispensable (which appear to mean the same thing) because they cannot be synthesised 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:

$$\text{DIAAS} = 100 \times \frac{\text{mg of digestible IAA in 1g of the test food}}{\text{mg of the same amino acid in 1g of the reference food}}$$

$$= 100 \times \frac{\text{TIAAD for test protein} \times \text{amino acid content of test food}}{\text{TIAAD for reference protein} \times \text{amino acid content of reference food}}$$

The DIAAS of the first limiting indispensable amino acid (i.e. lowest DIAAS) can be considered the overall DIAAS for the test food. Table 5 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.

Table 5. Digestible indispensable amino acid scores (DIAAS) for 14 protein-rich foods as measured in growing male rats, adapted from Rutherfurd, et al. (2015)

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

Combining corn-based breakfast cereal with bovine milk overcomes low lysine digestibility (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 as a website called vProtein. 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 at present; Massey University in New Zealand and Wageningen University & 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. Sustainability can be viewed in terms of footprinting for greenhouse gases, water, energy, social impact, distance to market or other variables; there is no consensus about what sustainability is, nor is there likely to ever be consensus. 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 metabolised 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 is important, i.e. the DIAAS.

A recent high-profile study by Poore and 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 Litre' standardised at 3.3 % protein for bovine and soy milk. This failed to recognise 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 6, in which the 'per 100 g protein' or 'per Litre of soymilk/bovine milk' data of Poore and Nemecek (2018) are adjusted for protein quality by dividing by published DIAAS values, where suitable DIAAS data are available.

Table 6. Estimated greenhouse gas emissions in kg CO_2eq per litre (milk, soy milk) or per 100g protein (all others) for production of protein-rich foods, as reported by Poore and Nemecek (2018). 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).

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

* units are kg CO_2 equivalent / Litre

[a] 10th percentile
[b] 90th percentile
[c] value for beef, as calculated using true ileal digestibility in pigs and reference requirements for 6-month to 3-year-old children (Ertl, et al. 2016).
[d] DIAAS for milk protein concentrate using digestibility measured in pigs and reference requirements for children 3 years and above (Mathai, et al. 2017).
[e] average between DIAAS values of soy protein isolate and soy flour (Mathai, et al. 2017).
[f] 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
[g] value for cooked peas (Rutherfurd, et al. 2015).
[h] value for cooked kidney beans (Rutherfurd, et al. 2015).
[i] average between DIAAS values for milk protein concentrate and skim milk powder (Mathai, et al. 2017).

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 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.

Adjusting footprint data for DIAAS does not change the overall conclusion that producing red meat has a higher environmental impact than for other protein-rich foods. However White and Hall (2017) pointed out that animal agriculture makes a number of unseen contributions to society. Animals can process human-inedible agricultural byproducts into edible materials, and their manure reduces the need for synthetic fertiliser.

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 modelled by van Zanten, et al. (2016), who proposed a soil-specific 'Land Use Ratio' to express the efficiency of plant- vs. animal-based cultivation. The higher micronutrient content and bioavailability in animal-based foods (Ertl, et al. 2016) somewhat counteract higher production inputs.

White and Hall (2017) modelled the scenario in which animal agriculture is eliminated from the US, but substantial reductions in environmental impact can also be made by modifying farming practices. Poore and Nemecek (2018) noted that environmental impact data were often skewed by a minority of high-impact producers, which highlighted an opportunity for targeted mitigation. 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 commercialisable. Food ingredients wholesalers typically will not stock a new protein ingredient until it fulfils 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 and insect-derived proteins are discussed below; unfortunately there is not space to examine other promising new food proteins such as algal protein, potato protein and leaf protein.

Cultured meat

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

> *"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."*
>
> The Future of Biology
> From 'Possible Worlds and other essays'
> J.B.S. Haldane, 1927

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). Cultured meat should be distinguished from meat analogues (also known as meat mimics, meat alternatives, imitation meat or mock meat) which have meat-like texture, colour and flavour 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):

- 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 vasculatory system
- Biophysical, biochemical and electrical stimulation, without which muscle cells do not mature properly.

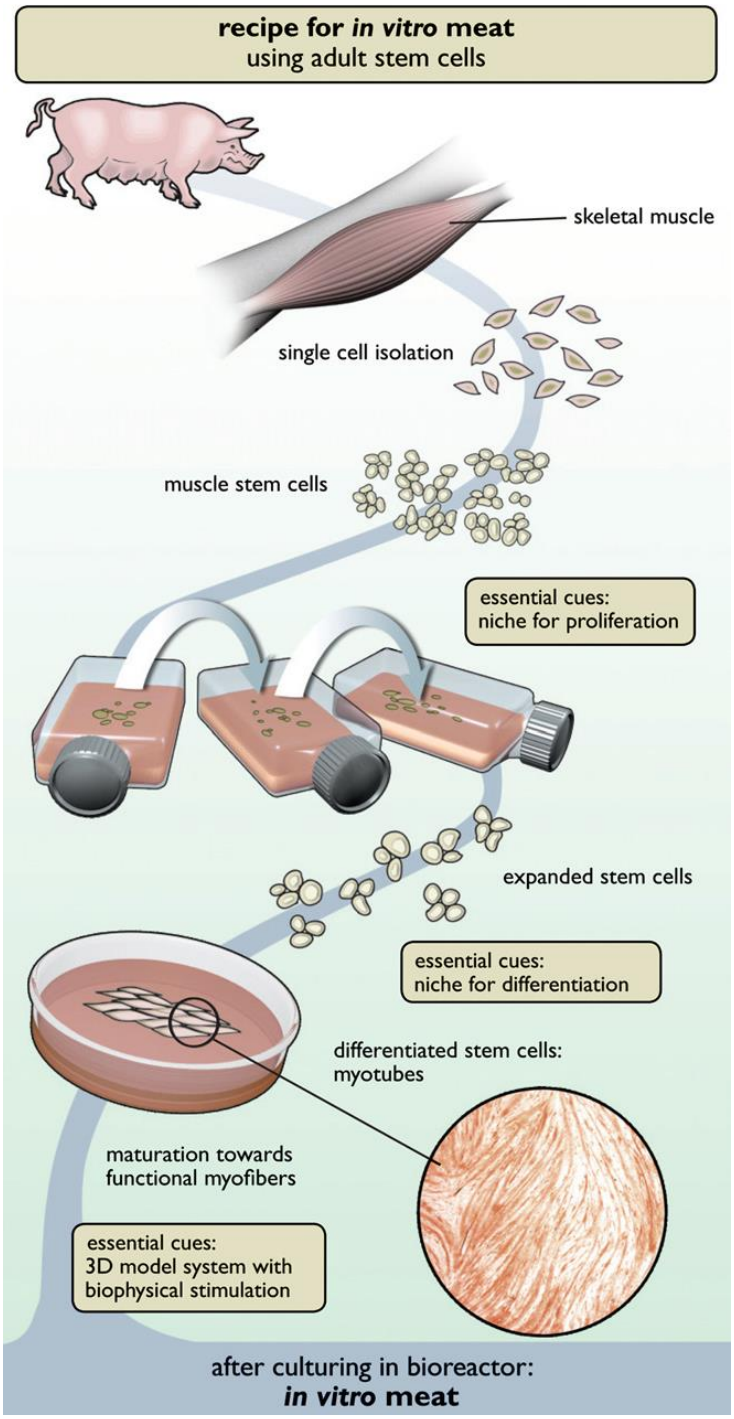

Figure 8. Schematic depiction of cultured meat production, from Langelaan, et al. (2010).

It may be possible to overcome some of these challenges by co-culturing 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 flavour and juiciness of meat, and adding adipocytes to the co-culture (Hausman and Poulos 2005) or when forming finished products (Post 2018) may improve the sensory properties of cultured meat.

Scaleup of muscle cell culture to large-scale production poses particular challenges in the differentiation and maturation phases (Figure 8), 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 micrometres 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 growth on microcarrier beads, cultivation as cell aggregates or immobilisation of cells in packed-bed reactors (Moritz, et al. 2015). Other possibilities include electrospun fibres, micropatterned surfaces or 3D printed scaffolds, potentially composed of edible materials (Datar and Betti 2010).

The resource-intensity of cultured meat production is difficult to gauge because large-scale production has not yet been realised. A lifecycle analysis of cultured meat production was attempted in 2011 (Tuomisto and 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 highlighted likely higher energy use in cultured 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.

At the time of writing cultured meat product research is attracting vast amounts of public interest and venture capital (Dance 2017), and producing very little scientific literature, probably because it mainly occurs 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 flavour similar to that of minced beef. However tissue engineering challenges mean that cultured meat products replicating the appearance, aroma, mouthfeel and flavour of whole-muscle meat cuts are still a long way from reality. The regulatory status and labelling 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 industrialisation of insect rearing and processing for food is relatively new. A wide variety crickets, locusts, grasshoppers, caterpillars, beetles, ants, fly larvae etc. can potentially be consumed for food (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, lipids and micronutrients, particularly 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). There is currently little known about the bioavailability of insect-derived nutrients for humans. In vitro protein digestibilities of insect proteins in the range 77-98% have been reported (Ramos-Elorduy, et al. 1997) and rat faecal digestibility of honey bee 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-coloured product, but aqueous-extracted cricket protein had better emulsifying and foaming functionality.

Aqueous extracts from freeze-dried powders of five insects were produced and characterised by Yi, et al. (2013). The protein makeup (SDS-PAGE) as well as 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 quite strong gels at 15% w/v.

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

Insect exoskeletons are comprised primarily of chitin, a polymer of N-acetyl-D-glucosamine. In one case alkali extraction of honey bees removed chitin and improved the faecal digestibility of protein in rats (Ozimek, et al. 1985), though causality was not proven. The corollary of this result is that chitin may inhibit protein digestion. On the other hand, 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 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 is 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).

Table 7. Protein, fat and energy content of selected insects, adapted from Dobermann, et al. (2017) with additional data from sources indicated in footnotes.

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	
Isoptera (termites)	35.34	32.74	
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 ricinii (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 purpuracens (chapulin adult)	61.33	11.7	404.22
Ruspolia differens (brown longhorn grasshopper)	44.3	46.2	
Skim milk powder[a]	37.3	0.80	373.8
Whey protein isolate[b]	92-96.1	0.4-1.0	
Soy protein isolate[c]	92.9	3.57	353
Raw beef[d]	81.2	14.1	454.9

[a] USDA Food Composition database entry 01091: Milk, dry, nonfat, regular, without added vitamin A and vitamin D
[b] from Foegeding, et al. (2002)
[c] USDA Food Composition database entry 16122: Soy protein isolate
[d] USDA Food Composition database entry 23427: New Zealand manufacturing beef, raw

Table 8. Regulatory status of insects as food and feed in 2017. From 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 lift 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.

The regulatory status of insect-based foods is summarised by jurisdiction in

Table 8. Due to 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).

Conclusions and future prospects

It is a time of complexity and paradox – the food supply is highly dynamic and globally interconnected, emerging protein sources present unknown opportunities and risks, epidemics of obesity and hunger coexist – but the need for delicious, nutritious and safe food is unchanged. In affluent markets, food choice is increasingly seen as a public demonstration of personal and tribal values, to the extent that the ethical, environmental and philosophical values embodied in food products can become more important than their sensory appeal or nutritional value. In the face of societal and technological change, the fundamentals of protein chemistry, biophysics and human physiology remain the best platform for responsible innovation.

Conflicts of interest

None.

Summary points

- Food proteins supply essential amino acids and play technological roles in foods.
- Nutritional and technological properties depend on protein source, extraction and purification, modification during food manufacture and interactions with other food proteins.
- Nutritional quality includes both the content of essential amino acids and their true ileal digestibility.
- Environmental sustainability comparisons should include protein quality measures.
- 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.
- Many insects are rich in protein, and with sufficient attention to hygiene and toxicity considerations they could become a mainstream source of food protein.

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