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Plant-based protein processing and undesirable consequences of food processing: a focus on protein quality, sulfur amino acid bioavailability, and quantification

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The nutritional quality of plant-based proteins (PBPs) is increasingly scrutinized as global dietary trends shift toward sustainable alternatives to animal-based proteins. This review critically evaluates the impact of food processing on essential amino acids (EAAs), with a particular focus on sulfur amino acids (SAAs), including cysteine and methionine. While processing techniques such as extrusion, autoclaving, fermentation, and high-pressure treatment can enhance the digestibility, functionality, and sensory properties of PBPs, they also induce undesirable chemical modifications. These include oxidation, racemization, and crosslinking, which reduce the biological availability of SAAs and compromise protein quality. Evidence from recent studies highlights significant losses of cysteine during processing, often attributed to the Maillard reaction and oxidative stress. Conventional quantification methods, such as performic acid oxidation, may overestimate SAA content due to the presence of pre-existing oxidized forms in processed foods. Alternative approaches, including Ellman's reagent-based assays, offer more accurate assessments of reactive cysteine and are better suited for evaluating true bioavailability. This review highlights the need for enhanced analytical methods to differentiate between total and biologically available SAAs, particularly in processed PBPs. Accurate quantification is crucial for refining protein quality metrics, informing food formulation, and developing correction factors to compensate for nutrient losses resulting from processing. Addressing these gaps will support the development of high-quality plant protein products and inform strategies to optimize processing conditions, ultimately enhancing the nutritional value of plant-based diets.

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Sustainability spotlight

This review contributes to sustainable food innovation by addressing how processing affects the nutritional quality of plant-based proteins. Plant-based proteins offer significant sustainability advantages, including lower greenhouse gas emissions, reduced land and water use, and fewer ethical concerns compared to animal-based sources. However, processing can compromise key nutrients, particularly sulfur amino acids (SAAs) like cysteine and methionine, through oxidation and structural changes. The paper advocates for improved analytical methods to accurately assess amino acid bioavailability, enabling the development of correction factors and optimized processing strategies. These advancements are essential for producing nutritionally complete, high-quality plant protein products that support global goals for climate resilience, resource efficiency, and public health.

1 Introduction

Proteins are nutritionally vital macromolecules composed of amino acids (AAs), which play a significant role in human growth and maintenance. The human body is incapable of synthesizing nine AAs known as essential/indispensable amino acids (EAAs/IAAs) either entirely or in sufficient amounts out of the twenty AAs encoded within the human genome. The remaining eleven AAs are known as non-essential/dispensable amino acids. Therefore, consuming food proteins that consist of all the EAAs in required quantities is crucial.[1] However, the daily protein requirement and protein metabolism vary from one individual to another. According to the WHO/FAO/UNU, the estimated daily protein requirement for a healthy individual is 0.66 g kg^{-1} body weight per day.[2] Since each protein contains a different amount of indispensable and dispensable AAs, various methods have been established over time to evaluate protein quality. Protein efficiency ratio (PER), protein digestibility-corrected amino acid score (PDCAAS), and

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digestible ileal amino acid score (DIAAS) are prominent examples, with DIAAS being the most recent.[3]

The world primarily relies on animal-derived and plant-derived proteins to meet its dietary protein needs. Although animal-based proteins (ABPs) have a higher demand globally, the current trend is shifting towards consuming plant-based proteins (PBPs). This is primarily due to the growing population, which makes it impossible for ABPs to meet the global demand for protein solely.[4] Despite the easier digestibility of ABPs within the human body, PBPs, which are less susceptible to enzymatic degradation due to their rich fibre content, have gained strong popularity among consumers due to their positive health aspects, including anticancer activity,[5,6] anti-inflammatory activity,[6,7] and a reduction in cardiovascular diseases.[6,8,9] PBPs also have a minimal environmental impact compared to conventional animal farming in terms of greenhouse gas emissions, deforestation, and high feed and water usage.[10,11] The current market is full of plant-based products, including meat analogs such as frankfurters, sausages, nuggets, and meatballs. However, ill characteristics of PBPs such as unbalanced AA composition, high fibers and carbohydrate content, poor palatability, poor solubility, presence of anti-nutritive factors (ANFs), low emulsifying, foaming, gelling capacities as well as low water and fat binding capacities means that processing is needed to improve their nutritional profile, protein digestibility, functional properties and sensory properties.[12–14] Heat,[15] high pressure,[14] and chemical treatments are highly used in the food industry for food processing. Even though food processing provides numerous advantages, it also has some drawbacks, which affect the nutritional quality and

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James D. House

Dr James Duncan House (1967–2024) was an internationally respected nutrition scientist at the University of Manitoba. He earned his PhD from the University of Guelph and built an influential research program in protein quality, sulfur amino acid metabolism, and sustainable protein systems. His achievements were recognized with numerous honors, including the Earl Willard McHenry Award, the highest distinction of the Canadian Nutrition Society (CNS), where he also served as President. In 2023, he was appointed the Manitoba Strategic Chair in Sustainable Protein. Dr House was a beloved teacher and administrator, known for his willingness to help and warm demeanor.

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bioavailability of some vital AAs[16–19] in food proteins. Proteolysis, protein crosslinking, racemization, and oxidation reactions are some of the negative consequences of food processing. This review focuses on the oxidation of sulfur amino acids (SAAs) during food processing, as the reactive side chains of SAAs are susceptible to oxidation during processing, rendering them unavailable for absorption and utilization in metabolic functions. Among the two SAAs, priority will be given to cysteine because there has been a considerable lack of studies on the oxidation of cysteine to cysteic acid in recent years. Performic acid oxidation, carboxymethylation, reaction with Ellman's reagent,[20] and pyridylethylation are already established methods for determining cysteine. However, Ellman's reaction seems more popular and effective due to its speed, efficient derivatization, and high efficiency, whereas other methods suffer from overestimation, incomplete reaction, and time consumption. Also, Ellman's reaction can be monitored *via* UV spectrophotometrically and chromatographically. This review distinguishes itself from recent scholarly literature on food protein processing and AA bioavailability by concentrating on the biochemical fate and bioavailability of SAAs in plant-derived proteins. While existing reviews primarily examine overall protein digestibility, structural modifications induced by processing, and broad metabolic or health outcomes, they seldom investigate SAA-specific degradation, oxidation, or transformation pathways in sufficient depth. For example, Li *et al.*[21] analyze structural alterations and digestibility differences across plant and animal proteins but do not provide an SAA-centric perspective on how processing affects cysteine and methionine chemistry. In contrast, this review explicitly outlines how thermal treatment, high-pressure processing, and related techniques may impair SAA bioavailability through mechanisms such as oxidation, Maillard reactions, and disulfide bond formation, aspects largely absent from prior studies. Similarly, Paoletti *et al.*[22] address metabolic availability without assessing the impact of plant-protein processing on SAA integrity, while Moyo[23] emphasizes nutrient retention with limited mechanistic detail at the AA level, particularly regarding the susceptibility of SAAs to heat, oxidative environments, and pH variations. Furthermore, the review identifies key research gaps, including the lack of recent comprehensive studies linking structural factors such as disulfide-bond density and matrix complexity to SAA accessibility and utilization. It also highlights the limited comparative analysis of emerging processing techniques, such as ultrasound, with respect to their effects on SAA fate, alongside ongoing analytical challenges in accurately quantifying post-processing SAA losses and differentiating between truly bioaccessible fractions and chemically transformed pools. Overall, this review advances the field by integrating SAA-specific chemical pathways, mechanistic insights into processing-induced transformations, the vulnerabilities of various plant protein sources, and measurement considerations into a cohesive framework, thereby informing strategies to optimize DIAAS, PDCAAS, and metabolic availability through processing advancements.

2 Plant-based proteins

2.1 Different types of plant-based proteins

The three primary sources of plant-derived proteins are legumes, cereals, and oilseeds. Legumes or Fabaceae belong to the Leguminosae family.[24] Legumes are considered to be a significant part of human nutrition and health as they are abundant in protein as well as vitamins[25] and minerals.[26] Therefore, legumes are broadly grown and consumed worldwide, especially in Africa and the Pacific. Protein levels in legumes differ by species, and the edible seeds from leguminous plants are referred to as pulses. Globulin and albumin are the two primary proteins in legume seeds. Legumes exhibit potential antioxidant activity due to their content of several bioactive components, including phytic acids, phenols, and tannins.[27] Despite being a rich source of protein, the ANFs in legumes reduce the bioavailability of nutrients. However, these plants contain rhizobium bacteria, which utilize nitrate (NO_3^-) and convert it into nitrogen (N_2).[28] The next few paragraphs will discuss some prime examples belonging to each of these PBPs categories.

Pea is a prime member of the legume family, owing to an annual global production of around 13.5 million metric tons. Genetic and environmental factors decide the protein content in peas. The amounts of AAs such as lysine, valine, arginine, and methionine are higher in peas,[24] whereas cysteine and glutamic acid quantities are poor. In addition to protein, peas are rich in fiber, vitamins such as C and E, and minerals including zinc, copper, manganese, nickel, and iron.[29,30] Polyphenols and saponins are two phytochemicals present in peas, which provide antioxidant activity and anticarcinogenic properties, respectively. Furthermore, both phytochemicals show anticarcinogenic properties as well.

Soybean protein, a type of legume, is also rich in protein. It contains all the EAAs, such as lysine, isoleucine, phenylalanine, tyrosine, threonine, tryptophan, histidine, and valine, including the SAAs methionine and cysteine. Protein, carbohydrates, edible oils, and minerals such as chloride, magnesium, copper, iron, silicon, and sulfur are the primary nutrients that make soy protein a nutrient-dense food. The protein content is 36%.[24,31] The primary limitation of soybean protein is that the lipoxygenases, saponins, and isoflavones in the protein cause an unpleasant, bitter, and puckering taste.

Pigeon pea, a popular legume in South Asia, is another primary member of the legume family enriched in proteins and other nutritional benefits. Red gram, congo pea, and no-eye pea are some alternative common names used to refer to pigeon peas,[28] and they owe their popularity to their outstanding survival capabilities in harsh conditions, such as heat, drought, and low and high yields, even in these conditions. The special character of pigeon peas is that they contain SAAs, both methionine and cysteine, in comparatively higher amounts,[32] whereas in other major legumes, the SAA content is limited. In addition, it contains higher amounts of aspartic acid, leucine, lysine, arginine, and glutamic acid. Phenolic acids, flavonoids, tannins, saponins, and phytic acids are the most common

phytochemicals present in pigeon peas, providing antioxidant, antidiabetic, and anti-inflammatory activities.[9]

Cereals are monocotyledons of the Poaceae family,[24] and over 60% of the food supply is satisfied by cereals around the world. Cereals comprise a significant portion of the population's diet due to their varied nutrient content, including proteins, carbohydrates, dietary fiber and vitamins.[6] Therefore, cereals are known as a staple food. However, the protein quality of cereals is considered to be reduced as they do not contain sufficient amounts of EAAs, such as lysine,[9] tryptophan, and threonine. In contrast to most legumes, cereals are composed of more methionine and cysteine, allowing complementation of lysine-rich, poor sulfur-containing legumes.[6,9,24]

Wheat (*Triticum aestivum*) is a significant member of the cereal family and consists of carbohydrates, protein, vitamins, and minerals.[33] Wheat is rich in SAAs but lacks lysine. Glutenin and gliadin are the principal storage proteins in wheat, collectively known as gluten. They are responsible for most of the viscoelastic properties in wheat, which facilitates the production of wheat-based food products, including leavened and unleavened bread, noodles, and pasta.

Rice (*Oryza sativa*) is another major cereal crop that belongs to the Poaceae family, also known as the grass family. India and China account for more than 50% of the global rice demand,[34] and rice is prevalent in both developed and developing nations. It is mainly consumed in the form of whole or broken kernels. Rice protein is nutritious, hypoallergenic, and healthy for human consumption. There are several protein types present in rice, namely albumin (4–22%), globulin (5–13%), prolamin (1–5%), and glutelin (60–80%), which is the major protein.[34] Rice seed is rich in lysine; comparatively, it is low in protein when compared to soybean or pulses.

Corn, or maize (*Zea mays*), is the third most widely cultivated staple cereal grain, after wheat and rice, in Asia, Africa, and Latin America. Corn contains starch (62%), protein (6–12%)[35] as well as a small fraction of vitamins and minerals.[36] Zein is the key storage protein in corn,[35] and a member of the prolamin family. Additionally, zein has garnered commercial interest due to its ability to form tough, lustrous, hydrophobic, and oleophobic coatings.

Oilseeds encompass seeds and fruits rich in oil and other essential fatty acids.[24] They contain a balanced amount of carbohydrates, lipids, and proteins, and both the seed and the defatted meal of the oilseed are rich in proteins with a favorable EAAs composition, including SAAs.

Flaxseed (*Linum usitatissimum*) is one of the oilseed plants rich in lignans, lipids, proteins, fiber, carbohydrates, and micronutrients such as vitamins A, C, and E.[37] There are two types of flaxseeds: brown flaxseed and yellow/golden flaxseed. Both brown and yellow flaxseed have similar nutritional values, contents, and equivalent amounts of short-chain fatty acids. Aspartate, glutamate, and arginine are abundant in flaxseed protein, which comprises two primary protein fractions: salt-soluble high molecular weight and water-soluble low molecular weight. These fractions account for 18–22%[24] of the seed's weight, depending on the cultivar and geographic origin. Flaxseed also contains a significant amount of phytotoxic compounds, including phytic acid, protease inhibitors, and cyanogenic glycosides.

Peanut (*Arachis hypogea*) is also a popular oilseed plant,[38] especially in China. It is used to produce edible oil, and the remaining oilseed meal is a byproduct with 50–55% high-quality protein 24.

2.2 Plant-based proteins over animal-based proteins

Of the two main dietary protein sources available that fulfil the global protein requirement, PBPs are currently in the limelight. A primary rationale for this trend is the growing global population, which necessitates developing alternative protein sources to ensure food security and meet increasing global demand for protein.[4]

Additionally, nowadays, people strive to eat healthy food and seek healthier options. PBPs are good alternatives because they contain numerous bioactive compounds, including phenolic compounds and vitamins,[39] which are beneficial to human health.[5–8] Plants synthesize these secondary metabolites to defend themselves against predators and pathogens, as well as to attract pollinators. However, their therapeutic effects are beneficial to us as they can provide antioxidant,[7,40] hypoglycemic, anti-inflammatory,[7] and antitumor activities,[5] as well as antibacterial activities against pathogenic microorganisms.

Moreover, the presence of fiber, polyunsaturated fatty acids, oligosaccharides, and carbohydrates helps reduce the risk of cardiovascular diseases[8,47] and type 2 diabetes,[48,49] whereas the consumption of some ABPs increases the risk of cardiovascular diseases.[49,50] The comparatively minimal environmental impact and absence of major ethical issues related to PBP production[51] make people more inclined to consume PBPs. This is because greenhouse gas emissions, deforestation, animal welfare, and humane animal slaughter are some major environmental and ethical concerns associated with conventional animal farming.[4,10,11] The following section highlights that, despite these positive characteristics of plant proteins, their overall quality still requires enhancement.

2.3 Improving the quality of plant-based proteins

To be qualified as a good source of protein, it must meet the human AA needs and demonstrate effective digestion, absorption, and utilization for metabolic functions.[52] Most of the PBPs are nutritionally incomplete because they are deficient in several EAAs. Table 1 compares EAAs between plant and animal proteins, highlighting the lower quantities in plant proteins. Some cereal proteins lack tryptophan, lysine, and threonine, whereas some legumes lack SAAs. Protein blending or combining complementary protein sources to create a novel dietary protein with high quality is one way to enhance the protein quality of PBPs.[42,53,54] SAA-rich but leucine-deficient buckwheat blending with leucine-rich SAA-deficient bean is one example of such a protein.[42] According to Fenn *et al.*,[53] adding 2% soybean protein to wheat flour improves the nutritional value of wheat flour by 28% by compensating for its lysine deficiency. Additionally, Bekele *et al.*[55] demonstrated that blending sorghum with chickpeas is beneficial.

Table 1 Comparison of essential amino acid compositions between plant proteins and animal proteins[a]

		Amino acid composition g/100 g dry matter basis										
Plant source	CP %	Phe	Val	Trp	Thr	Iso	Met	His	Leu	Lys	PDCAAS	Reference
Chickpeas	19.93	1.07	0.77	0.24	0.64	0.69	0.28	0.52	1.38	1.19		41
Buckwheat flour	12.47	0.43	0.48	0.14	0.41	0.33	0.24	0.34	0.63	0.66	54.27	42
Black beans-cooked	22.88	1.59	1.13	1.10	1.07	0.91	0.23	2.07	1.83	1.68	67.54	43
Red lentil-extruded	26.86	1.33	1.18	1.18	0.96	0.96	0.22	0.79	1.88	1.81	63.01	43
Split green peas-cooked	26.24	1.31	1.04	0.26	1.01	0.87	0.19	0.65	1.96	1.85	50.00	44
Whole green lentils-cooked	26.27	1.38	1.15	0.72	1.11	1.38	0.21	0.70	1.23	2.13	62.80	44
Casein	86.78	4.99	5.64	1.00	4.47	4.50	2.65	2.55	9.23	7.51	100	44
Egg g/100 whole egg		0.64	0.79	0.19	0.59	0.66	1.04	0.29	1.04	0.82		45
Beef g/100 g		4.0	5.7	1.1	4.0	5.1	2.3	2.9	8.4	8.4		46
Pork g/100 g		4.1	5.0	1.3	5.1	4.9	2.5	3.2	7.5	7.8		46
Lamb g/100 g		3.9	5.2	1.3	4.9	4.8	2.3	2.7	7.4	7.8		41

[a] CP%; crude protein content, Phe; phenylalanine, Val; valine, Trp; tryptophan, Thr; threonine, Iso; isoleucine, Met; methionine, His; histidine, Leu; leucine, Lys; lysine, PDCAAS, protein digestibility-corrected amino acid score.

ANFs present in PBPs impair the protein digestibility and reduce the bioavailability of AAs.[56] Pulses encompass a diverse range of ANFs, including protease inhibitors.[57] Trypsin inhibitors and tannins are protease inhibitors that affect protein quality. Tannins reduce the nutritional quality of pulses by forming insoluble complexes with dietary proteins and digestive enzymes, thereby impairing protein digestibility and AA availability.[57] Therefore, removing ANFs to improve protein quality and digestibility is very important. According to previous reports, processing methods such as ultra-high temperature processing (UHT),[58] cooking,[59] microwaving, autoclaving, and boiling[60] can reduce the unfavourable effects of ANFs.

Functionality is as important as nutrition because proteins impart color, texture, odor, flavor, and appearance to food formulations.[61,62] For instance, the viscoelastic and dough-forming characteristics of wheat gluten are critical for imparting texture to baked goods, while the curd-forming ability of dairy proteins is due to the colloidal organization of casein micelles.[63] Moreover, the excellent foaming characteristics of egg white[64] are precisely the reason it is used as an ingredient in foods such as cakes, chocolate, and mousses. Compared to PBPs, ABPs exhibit excellent functional properties, including solubility, gelation, emulsification, foam formation, water and fat binding, viscosity, and film formation. However, PBPs currently play a significant role in the protein market but require improved functionalities to survive the competition against ABPs. Before proceeding to a detailed analysis of published research on plant protein processing, it is essential to understand the concept of food processing, its typical categorizations, and the methods available within each category.

3 What is food processing?

3.1 Food processing techniques

Food processing is the transformation of plant, animal, and marine materials into intermediate or final value-added food products that are safe for human consumption. Food processing is carried out to ensure a longer shelf life against biological, chemical, and physical hazards, while maintaining the wholesomeness of the food, supplementing the nutrients required for health, providing variety and convenience in the diet, and adding value.[65] Food processing ensures consumers have access to a wide range of consumable food products at all times. Moreover, food processing ensures the maximum utilization of resources required for manufacturing a product, in line with sustainability goals, throughout all stages, from production to consumption.

There are various food processing techniques, which can be classified into thermal and non-thermal techniques.[65,66] Heat application is.[41,67,68] Thermal processing can be categorized as blanching, pasteurization, and canning. Blanching is a pre-treatment carried out before canning and freezing to deactivate enzyme activity,[69] which catalyses changes in flavor, texture, and food color. It is a gentle treatment that uses temperatures below the boiling temperatures of water for 2–3 min or less.[65] In pasteurization, liquids are heated at 60–100 °C for less than 1 min [65,66] to kill pathogenic microorganisms. Canning is carried out at 121 °C for 15 min to remove one targeted organism, *Clostridium botulinum*.[69] During canning, other food spoilage microorganisms are also inactivated. Refrigeration/freezing and dehydration also come under thermal processing. However, unlike the above-described methods, which are carried out by means of heat application, refrigeration/freezing and dehydration are carried out by means of heat removal and water removal, respectively.[65,70] The idea behind refrigeration and freezing techniques is to control the growth rate of the microbial population adequately, thereby stopping food spoilage and increasing the shelf life of most perishables by keeping them fresh through the arrest of undesirable enzymatic reactions. Dehydration is one of the oldest, most cost-effective, and energy-efficient methods used to extend the shelf life of food products by inhibiting the growth of microorganisms and enzymatic reactions.[65] During dehydration, water is removed through evaporation, vaporization, or sublimation.

Examples of chemical methods are acidification,[71] fermentation[70] and the addition of sugar[70] or salt.[72] High pH conditions in food are prone to microbial contamination. Therefore, lowering the pH[71] in the medium by adding acids such as vinegar[73] and lactic acid[69] will help increase the shelf life by inhibiting the multiplication of microorganisms. Acidification can be carried out together with heating and refrigeration as well. Pickling is also a common example of acidification used for the processing of vegetables and fruits. Fermentation is another ancient and widely used method, in which friendly microorganisms, such as yeast and lactic acid bacteria (LAB), convert fresh food into chemically distinct, more nutritious, and better-tasting food with a longer shelf life, including bread, alcoholic beverages, yogurt, and cheese.[71,74] Adding salt and sugar helps preserve fish, meat, and fruits, which are then converted into jams, jellies, and marmalade.[75] In irradiation, ionizing radiation from radioactive sources such as 60 Co and 137 Cs[76] is used to inactivate bacteria and to stop insect infestation in food. High-pressure processing,[77] also known as high hydrostatic pressure and ultra-high-pressure processing, utilizes elevated pressures of up to 600 MPa, with or without the application of heat (up to 120 °C), to achieve microbial inactivation.[78] High-power ultrasound[77] processing or sonication is mainly used for liquid foods to inactivate spoilage microorganisms. It utilizes energy generated by sound waves of frequency above 16 kHz.[65] Both Sections 3.1.1 and 3.1.2 discuss how processing contributes to improving the quality of both PBPs and ABPs based on the previous work.

3.1.1 Plant-based protein processing. Processing techniques alter the functional, nutritional, and sensory characteristics, thereby increasing the nutritional value,[79–81] EAAs availability,[80] protein digestibility,[55,68,82] flavor, texture,[83] and palatability[84] of plant proteins. A recent study showed that extruded chickpeas have higher protein quality than the raw seeds. Additionally, baking is preferable for home preparation over cooking because cooking can lower protein quality.[41] Another study[68] has shown that protein digestibility and the AA composition alter depending on the processing method, specifically extrusion, cooking, and baking, in red and green lentils. According to the results, red lentils had the highest protein quality after cooking, while green lentils had the highest protein quality after extrusion. Nosworthy, M. G. *et al.*[82] carried out another similar study on yellow and green split peas to investigate how different processing methods, such as cooking, baking, and extrusion, affect the *in vitro* and *in vivo* protein quality of these peas. According to the results, they found that green split peas generally had higher protein quality than yellow split peas, due to higher PDCAAS and DIAAS values. Furthermore, cooking is better for yellow split peas, and baking is better for green split peas in terms of protein quality. Wang *et al.*[59] showed that cooking beans and chickpeas in water increases protein content significantly. Extrusion appears to enhance the protein quality of buckwheat flour and pinto flour,[42] as indicated by PER values, increased digestibility, and higher PDCAAS values compared to baking. Another interesting study by Bailey *et al.*[85] on russet potatoes found that the high moisture boiling methods help preserve the limiting amino acids; however, other cooking methods, such as microwaving, baking, and frying, caused reductions in the limiting AA histidine. Furthermore, frying exerted the most significant damage to the amino acid score (AAS).

As described above in Section 2.1.3, the adverse effects of ANFs on nutrient composition, availability, and metabolism in PBP sources are significant. According to the literature, processing techniques such as soaking,[86,87] germination,[87] extrusion,[88,89] autoclaving,[90] high hydrostatic pressure,[91] fermentation,[80] boiling and microwaving[90] can reduce the ANFs in food. Khattab and Arntfield[90] conducted a nutritional quality study of several legume seeds (cowpeas, peas, and kidney beans) by applying various physical treatments, including soaking, boiling, roasting, microwave cooking, autoclaving, fermentation, and micronization. It turned out that soaking can reduce trypsin inhibitory activity by 10.22–19.85%. Autoclaving and fermentation appeared to be the most effective methods in reducing phytic acid content, as they reduced the phytic acid content by 65.04–70.49% and 66.83–68.94%, respectively. Additionally, tannin was significantly impacted by all the treatments applied, with boiling resulting in the greatest reduction, followed by autoclaving and microwave cooking. Linsberger-Martin *et al.*[91] reported that phytic acid can be reduced by high pressure by up to 36% in peas and 11% in beans. Moreover, treating peas at 600 MPa and 60 °C increased protein digestibility of up to 4.3%, regardless of the duration, while beans showed an increase of 8.7% when subjected to the same conditions for 60 minutes. Rathod and Annapure[88] observed that extrusion was capable of abolishing trypsin inhibitors by 99.54%, phytic acid by 99.30%, and tannin by 98.83% without altering the protein content, thereby increasing protein and starch digestibility by up to 89% and 96%, respectively. Ma *et al.*[92] demonstrated that various heat treatments enhanced the protein digestibility of field peas by inactivating antinutrients, such as tannins and trypsin inhibitors, and denaturing proteins, thereby facilitating the access of gastrointestinal enzymes for hydrolysis. Furthermore, applying heat can also destroy α-galactosides, protease inhibitors, and lectins.

Processing also enhances plant protein functionality.[14,93,94] For instance, as demonstrated by Chao *et al.*[14] high-pressure treatment can significantly improve air bubble encapsulation and foam formation in pea protein. Similarly, heat treatments can modify the foaming properties of soybean protein, making it suitable for use in aerated food systems.[95] Ghumman *et al.*[79] showed that germination can increase the foaming capacity of lentils and horse gram from 16.1% to 23.3% and from 13.3% to 26.7%, respectively. Fermentation has been found to enhance the water hydration capacity and oil-holding capacity of pea protein-enriched flour,[13] opening up new possibilities for its application. Wouters *et al.*[96] reported that enzymatically hydrolyzed wheat gluten exhibits enhanced solubility and superior foaming properties when compared to unhydrolyzed wheat gluten. They proposed that wheat gluten hydrolysates could serve as a functional alternative to egg white proteins in meringues and potentially other food applications. Flour made from germinated lentils and horse gram has shown increased foaming and water absorption capacities.[79] All the evidence

from previously published works proves the importance of various food processing techniques in improving the quality of PBPs.

3.1.2 Animal-based protein processing. Despite being proteins with high biological value, ABPs also undergo processing to enhance their shelf life, digestibility, functional properties, and sensory properties, as well as to eliminate foodborne pathogens. For instance, liquid milk is pasteurized to extend its shelf life, as it is hard to maintain milk in its liquid form without spoiling.[97] Liquid eggs are another animal-derived protein that undergoes pasteurization to ensure microbial safety, mainly from *Salmonella*.[98] In egg processing, hydrogen peroxide (H_2O_2) is added along with heat as an alternative to pasteurization.[99] The presence of H_2O_2 is advantageous in two ways, as it acts as a bactericidal agent and allows the use of relatively lower temperatures. H_2O_2 owes its antibacterial activity to its effective oxidizing capabilities. According to Monfort *et al.*,[100] triethyl citrate is another possible additive that can be used in conjunction with pulse electric field (PEF) treatment, followed by heat treatment, as an alternative to traditional pasteurization. Apart from pasteurization, dehydration is another processing technique applied to eggs, as dried eggs are easier to handle and formulate.[101,102] Dried eggs are more convenient for making foods such as bakery goods, mayonnaise, and pasta. Dehydration can be carried out using spray drying, pan drying, or belt drying.

Under non-thermal food processing techniques, fermentation holds a prominent place in ABP processing, as it enhances flavor, nutritional quality, and shelf life.[97] One popular example is the conversion of liquid milk into various products, such as cheese and yogurt. Meat fermentation is another major application of fermentation, which involves natural microbiota accommodated in meat substrate or externally added starter cultures. LAB, including *Lactobacillus*, *Pediococcus*, *Enterococcus*, *Leuconostoc*, *Lactococcus*, and *Weissella*, are the main meat fermentative bacteria, and their preservation ability on meat substances comes from various antibacterial metabolites, such as lactic acid, acetic acid, ethanol, fatty acids, and H_2O_2.[97] According to previous work, ultrasound processing is another non-thermal technique that benefits not only the improvement of functional properties, such as accelerated salt diffusion[103] and enhanced emulsification properties,[104] but also microbial inactivation[105] and improved sensory properties[106] of meat.

3.1.3 Undesirable consequences of food processing on proteins. Sometimes undesirable structural changes occur during processing, resulting in foods with reduced protein quality, and making some vital AAs biologically unavailable.[65,107] This is because the food is exposed to heat[108] various chemical reagents,[109] oxidative environments,[110] and alkaline environments[111] during processing, and the extent of the negative effect depends on the specific conditions used. It has been found that high heat and high-pressure treatments can induce protein modifications *via* proteolysis, protein crosslinking, AA racemization, protein–polyphenol interactions, and other reactions.[15,112–116] Proteolysis happens when either endogenous or commercial enzymes are used as processing aids, which affects the texture of the product.[117] Protein crosslinking reduces protein digestibility by aggregating proteins and restricting access to digestive enzymes.[118] A disulfide bond between two cysteine residues is the most common example of crosslinking. Racemization occurs at non-neutral pH and increases with pH and temperature. It is more likely to occur during peptide cleavage upon heating;[119] therefore, it is common in products such as baked goods, cornmeal products, vegetables, and eggs, which undergo proteolysis. The problem with racemization is that it changes the stereochemistry of AAs by converting L-amino acids to their D-version.[120] Proteins containing D-amino acids are considered of lower quality due to their poor cleavage by regular proteolytic enzymes and limited uptake and utilization within the human body. In addition, high-temperature, chemical reagents such as H_2O_2 (ref. 121 and 122) and derivatives of unsaturated lipids generated during oxidative reactions[115] can modify cysteine and methionine residues. As mentioned above, H_2O_2 owes its antibacterial activity to its effective oxidizing capabilities. If a protein is exposed to H_2O_2, this property can have a negative impact on the protein because SAAs are oxidized into biologically unavailable forms. Several studies have found that under unfavourable conditions, cysteine converts into cysteic acid,[123,124] and methionine converts into methionine sulfone through methionine sulfoxide,[125] depending on the degree of oxidation.[20,24,30] SAAs are not the only AAs that are affected during food processing. Lysine is another common example.[119,126] An extensive review of the negative impact of plant-derived and animal-derived proteins is discussed below in 3.1.3.1 and 3.1.3.2, respectively.

3.1.3.1 Negative impact of processing on sulfur containing amino acids. Among the key AAs affected during food processing, SAAs hold a prominent place. Cysteine and methionine exhibit fundamental differences in their inherent oxidation sensitivities, which are dictated by their sulfur chemistry. The thiol group of cysteine is highly nucleophilic and directly reactive with a wide range of reactive oxygen species (ROS), interpreting it susceptible to rapid oxidation into sulfenic, sulfinic, and ultimately sulfonic acids, as well as disulfide bond formation and irreversible conjugation with Maillard reaction intermediates. These various pathways make cysteine particularly vulnerable to degradation induced by processing, especially under thermal, oxidative, or carbonyl-rich conditions, resulting in substantial reductions in nutritional availability. Conversely, methionine, characterized by a thioether group, displays comparatively lower oxidative reactivity and primarily undergoes a two-step oxidation process (from methionine to methionine sulfoxide to methionine sulfone). Methionine sulfoxide is often reversible through the methionine sulfoxide reductase system, thereby reducing bioavailability loss. Although methionine can be oxidized during processing, particularly under high-temperature or high-pressure conditions, the resulting effects are generally less complex and more predictable than those impacting cysteine. Collectively, cysteine is more oxidation-sensitive, structurally reactive, and nutritionally labile, whereas methionine's behaviour is more chemically stable.[125] Chang *et al.*[19] found that treating soybean protein isolate with various concentrations of H_2O_2 at two

temperature settings (40 and 90 °C), effectively converted methionine into its sulfoxide at 40 °C, with only small amounts of sulfone or cysteic acid produced. At 90 °C, cysteine underwent rapid conversion to stable cysteic acid, while methionine was similarly transformed into its stable sulfone form. Furthermore, the methionine sulfoxide content was minimal at 90 °C. Cooking treatments, such as boiling, were found to lower the total content of SAAs.[127] Similarly, a decrease of cysteine content was encountered upon autoclaving in soybean protein concentrate starting from 1.09 g/100 g at 0 min, 1.03 g/100 g after 10 min, 0.87 g/100 g after 30 min, 0.80 g/100 g after 120 min, and finally, the loss (0.67 g/100 g) was significant after 140 min of treatment.[128] These changes were attributed to the partial destruction of cysteine by the formation of alternative compounds, such as cysteic acid, hydrogen sulfide (H_2S), and dimethyl sulfide. No significant reduction in methionine content was observed. A recent study carried out by Duque-Estrada *et al.*[89] noticed that extrusion processing caused a loss of certain EAAs, especially SAAs, in textured vegetable proteins (TVP) made from pulse-rich protein blends, although any structural modifications that happened to these SAAs were not specified. Similar results were obtained by Bekele *et al.*[55] after extruding chickpea, sorghum, and chickpea–sorghum blended snacks. They found that chickpeas and sorghum lost 7% and 26% of cysteine, respectively. When it comes to the blends, they analyzed three different chickpea–sorghum blending ratios (50 : 50, 60 : 40, and 70 : 30), which exhibited losses of 19%, 8%, and 8% cysteine, respectively. All these cysteine losses were attributed to the Maillard reaction. The Maillard reaction is a chemical process that occurs when proteins and sugars are heated together, resulting in the formation of covalent bonds between them to create new compounds. The characteristic flavours and aromas of roasted meat, as well as the crust and darkening of the bakery products, are two common positive outcomes of the Maillard reaction.[129] In addition to influencing the sensory qualities of a food product, Fu *et al.*[130] have reported that the Maillard reaction also enhances the functional properties of food, including emulsifying activity and foaming properties.[131] However, the Maillard reaction is also known to be one of the prime reasons for the destruction of flavour and nutritional quality during food preparation and processing. Considering food proteins, lysine has the most reactive amino group, which is involved in the Maillard reaction. The severity of the Maillard reaction is primarily affected by temperature, duration, and pH.[123]

3.1.3.2 Negative impacts of processing on animal-based proteins. ABPs also face negative consequences during food processing. For instance, liquid milk loses 5–10% of lysine during in-bottle sterilization.[132] It was also reported that sulfhydryl groups decreased in heated commercial milk.[133] When liquid milk is clotted for cheese production using milk-clotting enzymes or organic acids, the SAA-enriched whey protein is removed, resulting in a cheese protein with reduced biological value.[132] Even though products such as yogurt and cheese have longer shelf lives compared to liquid milk, they may not provide all the nutrients that liquid milk is supposed to provide. Additionally, milk is more nutritious when consumed in its liquid form.

The high susceptibility of liquid eggs to high heat during pasteurization results in protein damage and impaired functionality.[102] If H_2O_2 is involved, the high oxidizing ability that makes it a good antibacterial agent might negatively impact the protein quality of eggs. Chang *et al.*[19] observed that when egg white solids and casein were treated with different concentrations of H_2O_2 at two different temperatures, 40 °C and 90 °C, methionine in all samples was readily converted to its sulfoxide at 40 °C, with little sulfone or cysteic acid formation. At 90 °C, cysteine was quickly transformed to stable cysteic acid, while methionine was transformed to its stable sulfone form. Methionine sulfoxide content was minimal at 90 °C. Slum and Schreuder[109] carried out a study on fish meal and casein to investigate the oxidation of SAAs when treated with 30% H_2O_2. According to the results, the majority of methionine has been oxidized to methionine sulfoxide in both proteins. However, in treated casein, 25% of methionine had been further oxidized to methionine sulphone. The H_2O_2 produced during meat fermentation might also negatively affect SAAs by converting them to their oxidized forms, leading to a deterioration of the protein quality of fermented meat.

Lund *et al.*[110] showed that high-oxygen packaging atmospheres tend to reduce meat tenderness and juiciness. They also seemed to promote protein crosslinking and reduce thiol content, suggesting protein oxidation. Ferreira *et al.*[134] studied the effect of common cooking methods, such as boiling, roasting, grilling, and microwave reheating, on chicken refrigerated for one week and two weeks. Their results indicated that all cooking methods caused protein oxidation, including tryptophan depletion, loss of thiols, protein carbonylation, and the formation of crosslinks. However, boiling seemed to be the most damaging to chicken proteins. A previous study observed up to 90% decreases in lysine and arginine in casein and mixtures of casein with different carbohydrates, such as starch, sucrose, and glucose, following heat treatments including autoclaving and baking.[114]

Even though food processing is intended to improve food in every aspect, the undesirable changes taking place during the process highlight the importance and necessity of knowing the truly available quantities of each AA in a food protein, which determines the protein quality. Table 2 summarizes the positive and negative consequences that food processing methods have on both plant and animal proteins. Section 4 briefly describes the PER, PDCASS and DIAAS methods, which can be used to determine the protein quality of food proteins.

4 Protein quality evaluation

4.1 Protein quality

The quality of a protein depends on its capability to provide a sufficient amount of EAAs required for normal human metabolism and growth. Quality implies the nutritional value of a food protein, which depends on the quantity and balance of each AA as well as on the ability of that protein to be digested by proteases, absorbed, and utilized for metabolic function.[135,136]

Table 2 Summary of processing technologies and their documented effects on SAA bioavailability

Processing technology	Food type	Mechanism affecting SAAs	Effect on SAAs	Key findings/references
Boiling/cooking	Plant & animal proteins	Heat-induced oxidation; Maillard reactions; leaching	↓ Cys, ↓ Met (varies by food)	Boiling reduces SAAs in soybean;[60] potato boiling preserves limiting AAs better than frying[85]
Autoclaving	Legumes, soy	High heat + pressure; promotes oxidation & Maillard pathways	Significant ↓ Cys; minimal ↓ Met	Cysteine drops from 1.09 → 0.67 g/100 g in soy concentrate[128]
Extrusion	Legumes, cereal blends	High temp + shear; promotes Maillard reactions	↓ Cys; ↓ Met (food-dependent)	Cys reduced 7–26% in chickpea–sorghum snacks;[55] SAAs decreased in pulse-based TVP[89]
Microwave cooking	Legumes	Heat-induced oxidation and antinutrient inactivation	Moderate ↓ SAAs	Reduces tannins & trypsin inhibitors but causes SAA losses[90]
Baking/roasting	Legumes, cereals	High dry heat → strong Maillard reactions	↓ Cys; ↓ Met	Higher SAA loss than boiling in several products[140]
High-pressure processing (HPP)	Legumes	Pressure-induced structural unfolding	Mixed effects: ↓ phytic acid improves digestibility, mild SAA oxidation risk	Phytic acid ↓ up to 36% (peas), ↑ digestibility up to 8.7%[91]
Hydrogen peroxide (H_2O_2) treatment	Egg, dairy, meat during fermentation	Strong oxidizing agent targeting thiol and thioether groups	Cys → cysteic acid; Met → sulfoxide/sulfone	At 40 °C: Met → MetSO; at 90 °C: Cys → cysteic acid, Met → sulfone[19,110]
Fermentation	Legumes, dairy, meat	Generation of H_2O_2 by LAB; acidification	Possible SAA oxidation; ↓ antinutrients (↑ digestibility)	H_2O_2 may oxidize Met/Cys;[109] reduces phytic acid & improves digestibility[80]
Irradiation	Various foods	Free-radical formation	Possible oxidation of sulfur residues	Can oxidize cysteine and methionine depending on dose (literature cited in[76]
Ultrasound processing	Meat, dairy	Cavitation → localized heating, radical formation	Potential oxidation; tends to be mild	Improves functionality; microbial inactivation; limited SAA data[103–106]
Dehydration (spray/pan/belt drying)	Eggs, milk, plant proteins	Heat + exposure to oxygen	Moderate oxidation of Cys; Met more stable	Functional impairment documented in dried eggs[99,101]

Proteins can be divided into complete proteins and incomplete proteins. Foods that provide all EAAs in the right amounts for bodybuilding are called complete proteins. Incomplete proteins also contain EAAs but some are at insufficient levels.[137] The current section focuses mainly on three different protein quality evaluation methods: PER, PDCAAS, and DIAAS. Out of the three methods, PER and PDCAAS are the only methods recognized by national regulatory authorities for assessing protein quality.[3] DIAAS is a newly proposed method that is yet to be accepted by any regulatory authority.[3]

4.2 Routine protein quality evaluation methods

4.2.1 Protein efficiency ratio (PER). PER is the first standardized method for routinely assessing protein quality in food proteins. The concept behind PER is to evaluate the weight gain of an animal, typically weanling rats, in relation to its adequate intake of 10% protein over an 8 day period.[138] The test diet is compared with a casein control diet with the same protein intake. The PER value of a particular protein is determined by dividing the weight gain of the rats by the amount of protein consumed over this period. To standardize PER across laboratories, the obtained PER value is adjusted relative to the PER of casein by multiplying the raw PER of the sample by 2.5, which is the PER of casein.[138] In Canada, PER is the method used for assessing protein quality. PER is a simple method; however, the assumptions involved, which are that the whole diet consumed is used for the growth of the rat and the discrepancies of SAA requirements between rats and humans, limit the use of this method.[139] The former assumption is the most disadvantageous to PER, as it does not allocate appropriate credit for the protein used for maintenance purposes. Also, for the same reason, PER values are disproportional to protein quality. For example, a protein with a PER of 2.0 does not necessarily indicate that it is twice as effective as a protein with a PER of 1.0.

4.2.2 Protein digestibility corrected amino acid score (PDCAAS). In the United States, an alternative method to PER, known as PDCAAS, is used as the official method for.[141] It was the first method accepted that employed an indirect measure of IAA bioavailability as total tract digestibility.[142] To calculate a PDCAAS value for a particular food protein, AAS is also required. AAS is calculated by comparing the first limiting AA content in the desired protein to that found in a reference pattern determined by the FAO. AAS of 1 and higher indicates zero deficiency in the respective AA, whereas the lowest AAS indicates the first limiting AA of the desired protein. PDCAAS is then determined as the product of AAS and the true fecal nitrogen digestibility.[142] True fecal nitrogen digestibility is determined using rats as the animal model. Like PER, PDCAAS also has some drawbacks. Rats,[143] leading to an overestimation of digestibility. However, incorporating wire-bottom cages can overcome this issue. PDCAAS requires the use of a reference pattern to calculate AAS, and the reference pattern used is that of a growing population, such as preschool children.[142] This is inaccurate because it does not reflect the protein requirements for the growth and maintenance of an adult. Consequently, protein quality value can be underestimated.

4.2.3 Digestible indispensable amino acid score (DIAAS). The DIAAS represents a novel approach designed to overcome the methodological limitations of the PDCAAS. DIAAS is determined based on the digestible indispensable amino acid content in 1 g of food protein and the indispensable amino acid (IAA) reference ratio. The digestible IAA content is determined by multiplying the milligrams of IAA in 1 g of food protein by the true ileal digestibility coefficient for the same dietary indispensable AA. The digestible IAA reference ratio is calculated by dividing the digestible IAA content in 1 g of food protein by the milligrams of the same dietary IAA in 1 g of the reference protein (amino acid scoring pattern). For a given reference protein amino acid pattern, the DIAAS is the lowest calculated value for the DIAA reference ratio, expressed as a percentage. A notable distinction between PDCAAS and DIAAS is that DIAAS utilizes the true ileal digestibility of individual EAAs, whereas PDCAAS relies on fecal digestibility. The determination of digestibility should ideally be based on the true ileal digestibility of each AA, preferably determined in humans. However, if this is not feasible, it should be determined by growing pigs or rats. The DIAA scoring pattern of each AA provided by the test protein is compared with the AA requirements of the reference population. In 2013, the FAO/WHO introduced revised AA scoring patterns that account for the varying AA needs of infants, young children, older children, and adults. DIAAS values can fall below or exceed 100%; unlike PDCAAS, values exceeding 100% are not truncated.

5 Reactive amino acids

5.1 Available *vs.* unavailable amino acids

Even if a particular food protein contains all the EAAs, some may not be biologically available. The term "availability" represents the amount of dietary AAs absorbed from the gastrointestinal tract in a chemical form appropriate for protein synthesis in the human body.[144] Therefore, the unavailability arises when the AAs do not exist in biologically available forms. For instance, several prominent AAs, such as lysine, cysteine, and methionine, exist as biologically unavailable lysinoalanine, cysteic acid, and methionine sulfone, respectively, especially in processed food proteins.[19,52,67] An interesting study by Anderson *et al.*[145] reported that in weanling rats, both cysteic acid and methionine sulfone were not utilized for growth and did not support their weight gain. However, methionine sulfoxide supported growth at only 60% of the efficiency of methionine. SjÖberg[124] also discovered that both methionine sulfone and cysteic acid were not completely available for rats. A similar study on growing chicks by Kuzmicky *et al.*[146] found that D-methionine sulfoxide was significantly less available than L-methionine sulfoxide. Section 5.2 below summarizes the contributions of bioavailable methionine and cysteine, particularly to transsulfuration and remethylation pathways.

5.2 Sulfur amino acid metabolism

Methionine is classified as an EAA, whereas cysteine is considered non-essential, provided that dietary methionine is

available in sufficient quantities. Therefore, the apparent requirement for methionine represents both methionine and cysteine together. In humans and animals, cysteine can only be synthesized from methionine through the transmethylation pathway (Fig. 1). Methionine reacts with adenosine triphosphate (ATP) and produces *S*-adenosyl methionine (SAM), which is an important methylating agent. When a methyl group ($-CH_3$) is transferred from SAM, *S*-adenosylhomocysteine is produced.[125,147] Once the *S*-adenosylhomocysteine is condensed with serine, it becomes cystathionine. Finally, cysteine is produced when cystathionine is cleaved by the enzyme cystathionase. Serine and methionine are the two AAs that provide the carbon skeleton and the sulfur (S) atom for cysteine, respectively. This entire process of conversion from methionine to cysteine is known as the transsulfuration pathway (Fig. 1). SAM serves as an important source of methyl and propyl amine groups for a broad range of compounds, including alkaloids, choline, creatine, epinephrine, n-methylated AAs, nucleotides, and polyamines as well as phospholipids, polysaccharides and nucleic acids.[147] Homocysteine can be remethylated to methionine with methyl donors, either methyl tetrahydrofolate or betaine. Therefore, the occurrence of remethylation is sensitive to folate status. Cysteine can undergo transamination to form thiopyruvate, which is subsequently desulfurated into pyruvate and hydrogen sulfide (H_2S). Cysteine can also be oxidized to cysteine sulfinic acid, which is then decarboxylated to hypotaurine. This intermediate is subsequently oxidized to taurine, which contributes to the formation of taurocholic acid, a key bile emulsifier involved in fat digestion. Another key role of cysteine is the synthesis of an important intracellular antioxidant known as tripeptide glutathione.[125,147,148] Cysteine availability is the limiting factor in glutathione synthesis, especially during conditions of malnutrition or inadequate dietary protein intake.[148] It is also necessary for regulating inflammatory cytokines and maintaining overall protein balance in the body. Therefore, consuming plant-derived proteins potentially results in positive nutritional effects on metabolism as they have a higher cysteine-to- methionine ratio than animal proteins (Table 3). However, despite the higher cysteine content in plant

Table 3 Methionine and cysteine contents of various animal and plant proteins (adapted from Masella and Mazza)[148]

Protein source	Methionine (g/100 g protein)	Cysteine (g/100 g protein)
Beef	2.5	1.4
Chicken	1.5	1.4
Salmon	2.9	1.1
Egg	3.2	2.3
Casein	2.9	0.7
Wheat	1.5	2.6
Pea	0.9	1.7
Lupin	0.7	1.7
Rapeseed	2.0	2.7

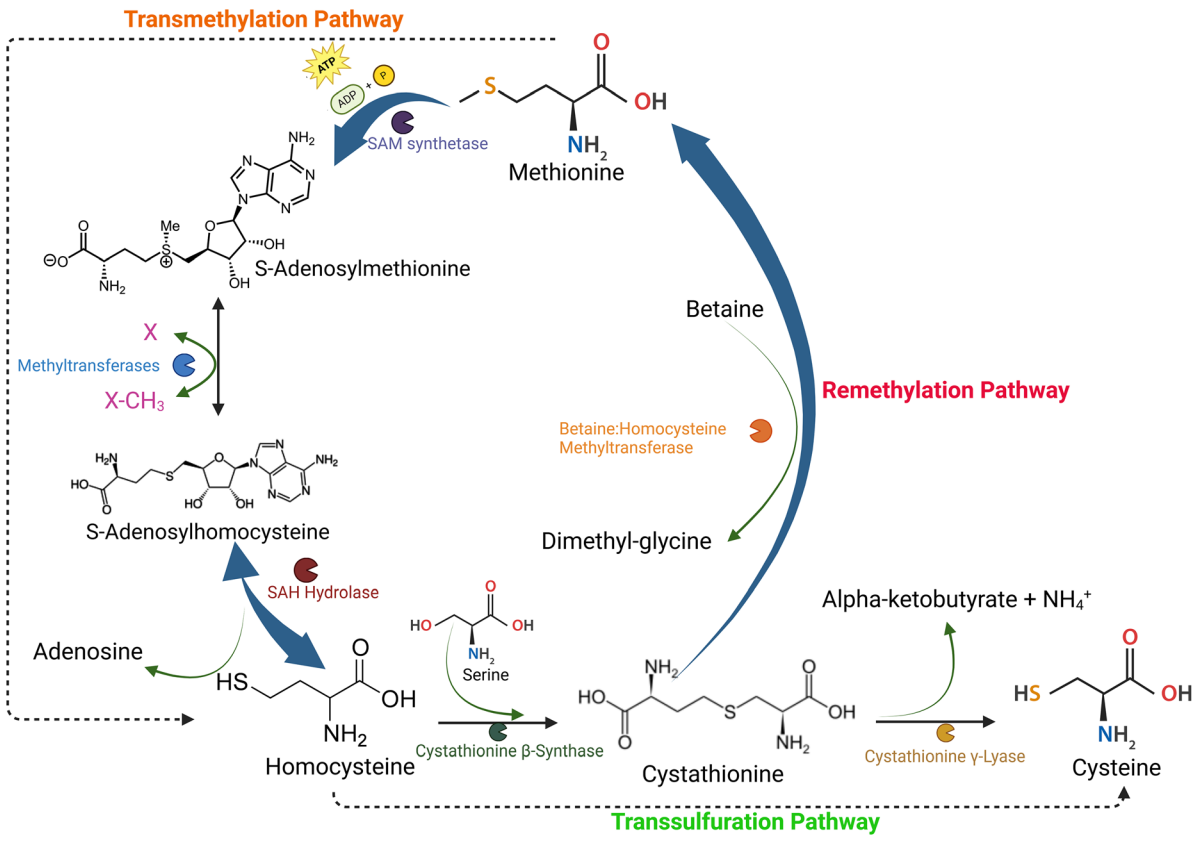

Fig. 1 Sulfur amino acid metabolism.

proteins, the challenge lies in the potential loss of cysteine during food processing, limiting the benefits of this "cysteine advantage" of plant-based proteins.

5.3 Measuring total amino acids

An essential characteristic of food is its capacity to satisfy the nutritional needs of consumers. Therefore, understanding the precise quantities of individual AAs provided by food proteins is critical for meeting protein requirements, as these values directly influence AA bioavailability in the human body. Particularly when the AA profile is altered during processing, the application of accurate analytical techniques for AA quantification becomes imperative. When considering the protein content and composition of a food protein sample, it can be achieved by either finding the total nitrogen (N) content and expressing it as 'crude protein' or analyzing the AA composition and then determining the sum. Knowing the total AA content in a protein sample is useful in many ways. For example, the identity of a protein can be determined based on its AA composition.

In addition, it also supports structure elucidation and detection of an atypical AA that might be present in a protein. The two basic principles of AA analysis are the hydrolysis of peptide bonds in the desired protein and the subsequent separation of AAs *via* chromatography. Usually, separated AAs are derivatized before analysis. Currently, various methods exist to evaluate the bioaccessible AA content in food proteins, which refers to the amount of AAs that are released from the food matrix during digestion and become available in the intestinal lumen for potential absorption. For most AAs, the AOAC standard approach is applied, which is commonly known as regular amino acid hydrolysis.[149] This method hydrolyzes the protein sample for 24 hours with 6 N HCl. Tryptophan and SAAs prefer different approaches because both tryptophan and cysteine are destroyed during the regular analysis. Methionine is also oxidized to its sulfoxide, and sulfone forms if oxygen is present during the regular analysis. Therefore, tryptophan is analyzed using the alkaline hydrolysis method,[149] whereas for SAAs, the performic acid oxidation method is applied. As mentioned above, after each hydrolysis, the usual method of separation and quantification involves chromatography techniques such as ion-exchange chromatography, high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), gas chromatography (GC), or GC coupled with mass spectrometry (MS).

5.4 Methods available for determining sulfur amino acids

The sections below discuss several methods for SAAs, with a primary emphasis on the quantitative analysis of cysteine.

5.4.1 Performic acid oxidation. Performic acid oxidation involves the oxidation of thiol and disulfide groups into cysteic acid with performic acid in the presence of H_2O_2.[149–151] The reaction mixture is sent for AA analysis after 16–18 hours of incubation, followed by acid hydrolysis. In ion-exchange chromatography, cysteic acid is supposed to elute in the third position. However, the elution position may differ depending on the specific instrument, precise temperature, molarity, and pH of the buffers. This reaction is not specific to cysteine but also influences methionine by converting it into either methionine sulfoxide or methionine sulfone. Hence, both cysteine and methionine in proteins are commonly assessed using the performic acid oxidation method and are mostly interpreted as total SAAs. Since an oxidation step follows, the total measurement approach does not facilitate the differentiation between the actual reactive cysteine (or reactive methionine) and cysteine (methionine), which has already been oxidized into cysteic acid (or methionine sulfoxide and methionine sulfone) in the initial protein prior to performing performic acid oxidation, especially in processed food.[149] In addition, the phenolic group present in tyrosine[149] is also susceptible to this reaction, and this side reaction can be minimized by adding phenol to the reaction mixture. Moreover, foods that have not undergone processing might also contain biologically unavailable AAs due to post-translational modifications. Therefore, this method is suitable for determining the total SAA content in a food protein, but not for assessing its true availability. Thera *et al.*[152] examined performic acid oxidation with the ultra-high performance liquid chromatography (UPLC) method to quantify protein-bound SAAs in zooplankton. They reported that both cysteic acid and methionine sulfone exhibited linearity from 5 to 250 pmol ($r^2 = 0.99$), with detection limits of 13 pmol and 9 pmol, respectively.

5.4.2 Carboxymethylation of cysteine using iodoacetamide/iodoacetic acid. The idea behind this reaction is to use a reagent that can block the thiol group of cysteine prior to quantification.[153] Both iodoacetic acid and iodoacetamide can block the thiol group by derivatizing cysteine into *S*-carboxymethyl cysteine with the introduction of acidic carboxymethyl (—O_2CCH_2S—) groups and *S*-carboxyamidomethyl cysteine, respectively. When iodoacetate is used, it introduces negative charges to the protein, whereas such divergence is unfavourable; iodoacetamide comes into the picture as it adds a neutral carboxyamidomethyl (H_2NCOCH_2S–) group to cysteine. For AA analysis, *S*-carboxyamidomethyl cysteine can be converted into *S*-carboxymethyl cysteine upon acid hydrolysis. If carboxymethylation is carried out before and after the reduction of disulfide linkages, the amount of disulfide linkages can also be quantified. The advantage of this method is that the reaction is irreversible with either of the reagents, ensuring the production of a stable final product for analysis. AA analysis can be carried out using HPLC. If both reagents are used simultaneously, varying the ratios to derivatize the cysteine residues, electrophoresis is an appropriate method to quantify the cysteine residues due to the charge differences of the products. However, the inability to assess the completeness of the reaction with either of the reagents is a disadvantage of this method.

5.4.3 Reaction of sulfhydryl group with Ellman's reagent. In 1959, Ellman introduced an aromatic disulfide reagent, 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), to estimate free thiol groups.[20] DTNB reagent, which is known as Ellman's reagent today, reacts with aliphatic thiols by an exchange reaction (Fig. 2(a)), yielding a mixed disulfide of the protein of interest and 1 mole of 2-nitro-5-thiobenzoate (TNB) per mole of protein

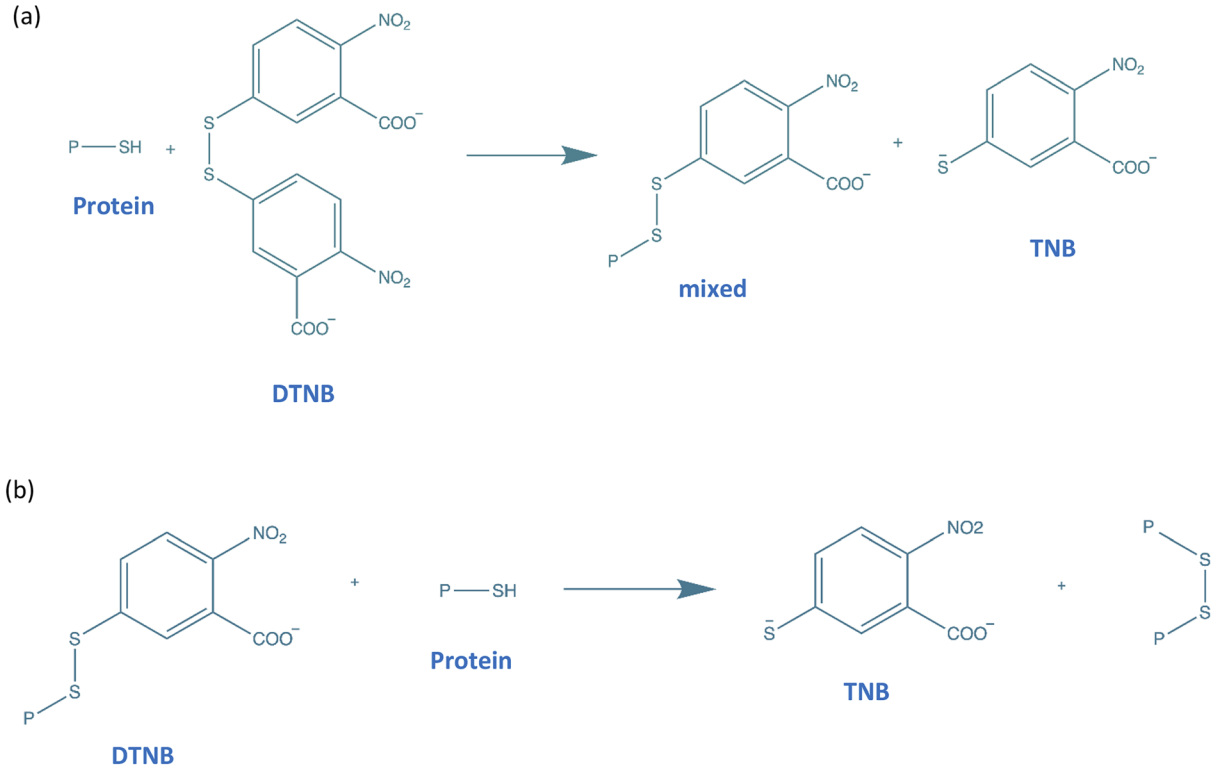

Fig. 2 (a) Ellman's reaction between DTNB and the thiol group produces the mixed disulfide between DTNB and the protein and TNB anion (b) formation of a disulfide linkage between two protein sulfide molecules instead of the mixed disulfide.

sulfhydryl group. Thionitrophenylate anion (TNB) exhibits an intense yellow colour with a molar absorptivity coefficient of 13 600 M^{-1} cm^{-1} at 412 nm. This method is simple, rapid, and easily applicable to routine measurements.[133,154] For instance, simple thiols like cysteine develop a complete color within 2 minutes and remain stable for 2 hours. Intramolecular and intermolecular disulfide formation is also a possibility during this reaction (Fig. 2(b)). Despite the reaction, stoichiometry remains the same, causing no barriers to analysis. Biyolojik *et al.*[155] studied oxidative stress in biological samples using sulfhydryl groups of protein, glutathione and free cysteine as indicators. Their aim was to apply the Ellman method for assay of sulfhydryl groups to a microplate scale and to determine this method's analytical performance, and reported that the limit of detection (LOD) and limit of quantification (LOQ) have been shown to be 1.06 and 3.52.M, respectively. Chen *et al.*[156] presented an assay for total thiols and total disulfides in biological samples (OVCAR-3 cells) *via* HPLC quantification of 5-thio-2-nitrobenzoic acid (TNB) derived from the reaction of thiols with Ellman's reagent.

A recovery study with OVCAR-3 cells revealed that the recovery yields for TNB in the procedures for determining non-protein thiols, protein thiols, non-protein disulfides, and protein disulfides were 99.4 ± 1.2% ($n = 3$), 98.1 ± 5.0% ($n = 3$), 95.6 ± 0.9% ($n = 3$), and 96.6 ± 2.3% ($n = 3$) respectively. They also quantified glutathione (GSH) *via* the measurement of the GSH-DTNB adduct (GSH-TNB). By using 326 nm as the detecting wavelength, the HPLC detection limit for TNB and the GSH-TNB adduct was determined to be 15 and 7.5 pmol, respectively.

There are a couple of limitations of the Ellman's reaction.[157] One is the tendency of the liberated TNB to autoxidize during prolonged incubation. Also, Ellman's reaction cannot be used for colored proteins such as hemoproteins, where the Soret band overlaps the 412 nm absorption maximum of the TNB anion. A study by Ou *et al.*[158] noticed difficulties in directly determining SH and –S–S– group content in soymilk because of the high turbidity and low SH group content of soymilk. In addition to these drawbacks, several concerns warrant attention.[159] Two primary factors influencing this reaction are pH and steric effects. The reaction specifically involves the thiolate form of cysteine, which is predominant at a pH around 8.5, the pK_a for cysteine thiol deprotonation. At pH values below 8.5, minimal thiolate is present, limiting reaction progress, whereas higher pH (pH 9.0 or above) favors thiolate formation but can cause rapid breakdown of DTNB, resulting in high background signals. Therefore, optimal conditions require a balance where cysteine residues are sufficiently deprotonated while maintaining reagent stability, with pH 8.0 historically recommended. To ensure full reaction of cysteine thiols, proteins should be denatured to expose sterically hindered residues, using agents like urea, guanidine, or detergents. Table 4 summarizes the SAA species measured using various analytical approaches, including their limit of detection (LOD) and limit of quantitation (LOQ) values.

Table 4 Comparison of analytical methods used to quantification of SAAs

Analytical method	SAA species	LOD	LOQ	Reference
Ellman's reaction-HPLC	Cysteine in human blood plasma	1.06 μM	3.52 μM	155
Performic acid oxidation-UPLC	Protein bound SAAs in zooplankton	Cysteic acid = 13 pmol Methionine sulfone = 9 pmol		152
UPLC	Cysteine (Cys) Cysteinylglycine (CysGly) Homocysteine (Hcys) γ-Glutamylcysteine (γ-GluCys) Glutathione (GSH) in pig tissue	Cys = 0.25 nmol g^{-1} CysGly = 0.21 nmol g^{-1} Hcys = 0.17 nmol g^{-1} γ-GluCys = 0.29 nmol g^{-1} GSH = 0.79 nmol g^{-1}	Cys = 0.76 nmol g^{-1} CysGly = 0.64 Hcys = 0.52 γ-GluCys = 0.88 GSH = 2.41	160
HPLC-fluorescence detection	Homocysteine methionine (Met) and cysteine (Cys) in blood	Hcys = 0.13 μM Met = 0.14 μM Cys = 0.13 μM	Hcy = 0.04 Met = 0.43 Cys = 0.46 μM	161
Rapid LC-MS/MS analysis	L-Methionine L-Cystine of cell supernatant from purified peripheral blood mononuclear cells	L-Methionine = 1 fmol L-Cystine = 1.25 fmol		162

5.5 Fate of other indispensable amino acids during food processing

As previously mentioned, apart from SAAs, other EAAs are susceptible to the negative effects of food processing. Lysine is one prominent example, and it is converted to lysinoalanine (LAL) during food processing.[115] LAL can form during alkaline treatments of food proteins when lysine reacts with dehydroalanine, which is produced by the β-elimination reaction from cysteine and serine. Furthermore, LAL can also be present in food that has been exposed to heat. Finley *et al.*[163] carried out an H_2O_2 treatment followed by an alkaline treatment on several different proteins, soy, safflower, and alfalfa leaf protein, to study the effect of cysteine oxidation on LAL formation. The oxidation state of cysteine appears to be a key factor in LAL formation, as no LAL was detected when cysteine was present in oxidized forms such as cysteic acid. Another study has shown that the oxidation of cysteine matters regarding Maillard products when exposed to heat.[164]

Several other studies have reported lysine losses in chickpeas, which is a lysine-rich protein source (6.5–6.7%). Autoclaving chickpeas at 121 °C for 30 and 60 minutes reduced the lysine content by 10% due to the longer exposure time at high temperatures. Moreover, this reduction is higher when the.[108] Alajaji and El-Adawy[127] also found that boiling and autoclaving cause lysine losses in chickpeas due to high heat. Besides chickpeas, the lysine content in album protein isolates from *Chenopodium album* diminishes when heated, as a result of crosslinking reactions and structural and conformational alterations caused by the heat exposure.[15] The lowest lysine availability occurred at 100 °C for 30 min, whereas the highest was at 80 °C for 15 min.

Tryptophan is another AA that undergoes the Maillard reaction with its indole group, especially at acidic pH.[165] Alajaji and El-Adawy[127] reported tryptophan losses during cooking treatments, although the reason was not specified. Simat and Steinhart[122] also reported that tryptophan is susceptible to oxidative agents such as H_2O_2. Upon heat application, threonine forms pyrazines, pyridines, and pyrroles, reacting with sugars.[144] In addition, histidine is also susceptible to oxidation, and 2-oxohistidine and hydroxyhistidine are some major products of histidine oxidation.[166,167] Dyer, J. M. *et al.*[168] observed histidine oxidation in lactoferrin after heat treatment in the presence of lactose.

The above sections (3.1.3, 3.1.3.1 and 3.1.3.2) on the negative consequences of food processing highlight the importance and necessity of establishing methods to determine the true reactive or biologically available quantities of AAs, leading to knowing the overall protein quality of food.

6 Conclusions and future directions

Processing plays a pivotal role in influencing the nutritional quality and bioavailability of PBPs, particularly EAAs and SAAs such as cysteine. While numerous processing techniques can enhance the digestibility and techno-functional properties of PBPs, they may also induce chemical modifications, including AA oxidation, that compromise overall protein quality. This concern is especially pertinent for plant-derived proteins, which inherently contain lower levels of SAAs compared to many animal-derived counterparts. Despite the recognized nutritional importance of SAAs, research on cysteine bioavailability, particularly in processed foods, remains limited and outdated. Although cysteine can be synthesized from methionine, it becomes conditionally essential when dietary methionine intake is inadequate, highlighting the necessity for precise, reliable assessment methods of SAA availability in plant-based diets. Current analytical approaches, such as performic acid oxidation, risk overestimating SAA content when oxidized species are already present within the food matrix, potentially obscuring actual nutritional value and skewing protein quality evaluations. As such, alternative analytical methods that circumvent pre-oxidation, such as Ellman's assay, are valuable for enhancing the accuracy of SAA quantification and refining protein quality scoring systems. Advancing the understanding of SAA bioavailability and improving the assessment of PBP nutrition will require coordinated efforts across research and industry sectors. Developing and validating oxidation-free

analytical protocols for the direct quantification of cysteine and methionine in processed foods are essential steps toward accurate SAA measurement. Standardization of interlaboratory methodologies to independently quantify reduced and oxidized SAA species will facilitate consistent comparisons across studies, while exploring correction factors for processing-induced oxidation may further improve the precision of protein quality assessments. Updating SAA bioavailability data through contemporary human and animal studies, reflecting current plant-based ingredients and processing technologies, is also crucial. Such research should investigate the impact of specific processing methods, including extrusion, fermentation, thermal treatments, high-pressure processing, and enzymatic modifications, on SAA retention and absorption. Equally important is the identification of processing conditions, such as oxygen exposure, temperature, pH, moisture, and antioxidant use, that can mitigate SAA degradation during industrial manufacturing. Building on this knowledge, innovative processing strategies, such as low-oxygen environments and enzyme-assisted stabilization, could help preserve cysteine and methionine content, with emerging technologies like cold extrusion, pulsed electric fields, and controlled fermentation offering promising avenues to enhance SAA stability in PBP ingredients. Finally, collaboration with regulatory agencies is necessary to update protein quality evaluation frameworks, including DIAAS and PDCAAS, to account for oxidative losses and bioavailable SAA fractions. Promoting SAA-specific labelling requirements and developing comprehensive reference databases documenting SAA content in raw and processed plant-based foods will provide essential tools for researchers, policymakers, and industry stakeholders committed to improving the nutritional profile and processing practices of PBPs.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

No primary research results, software, or code have been included, and no new data were generated or analyzed as part of this review.

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