Kasturba Health Society
-Medical Research Centre

PERSPECTIVES ON PROTEIN QUALITY

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Proteins in our diets

Ability to accurately & objectively define protein quality -an important role in addressing human nutrition requirements, nutrition policy, trade, product development.

Dietary proteins extremely diverse: notable variations in amino acid composition, digestibility between different sources of dietary protein

Broadly classified by origin (plant/animal), amino acid composition (essential/indispensable vs non-essential/ dispensable; complete vs incomplete).

Composition or quality of various proteins may be so unique that their influence on physiologica function in the human body can be quite different.

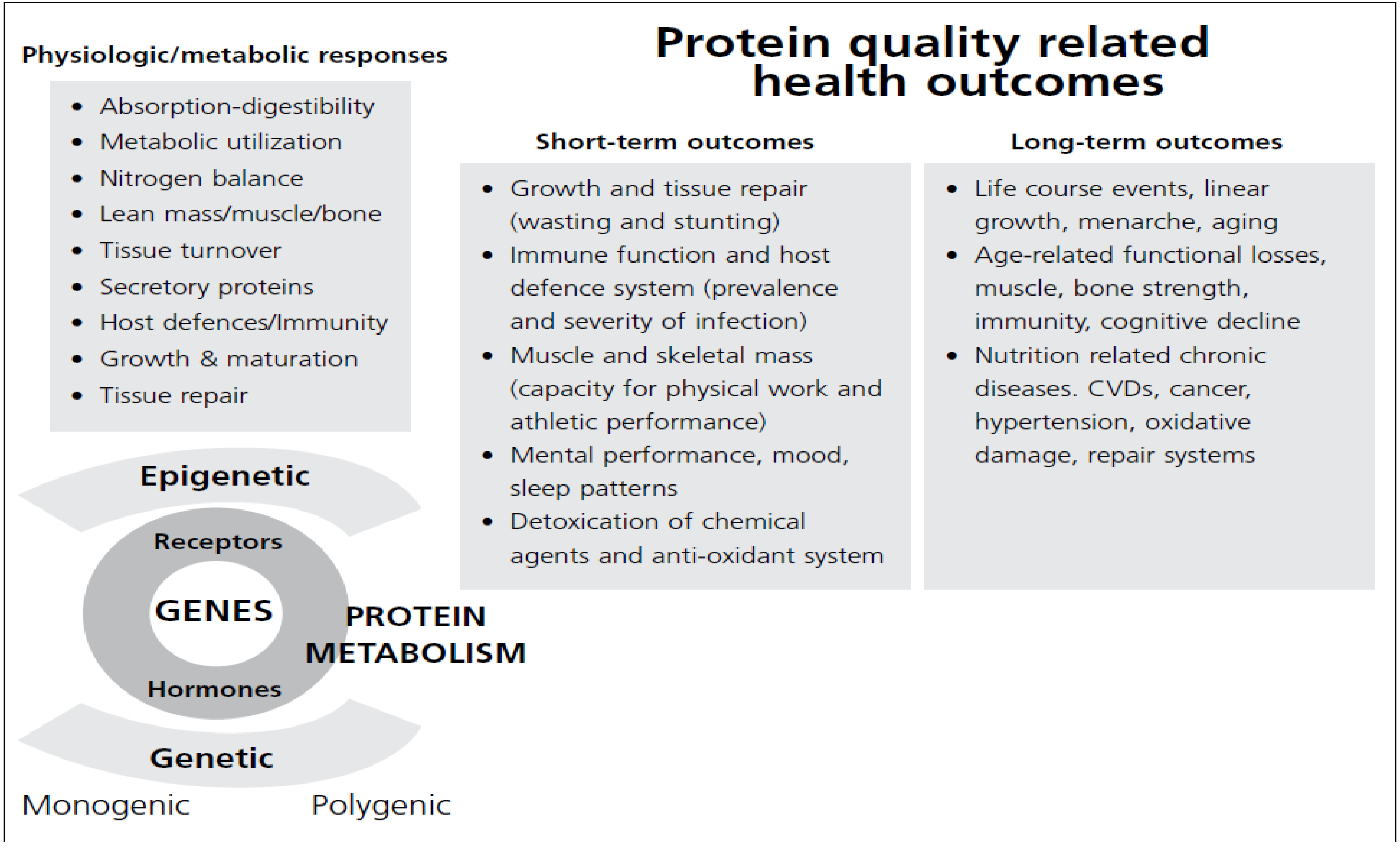
Both the right amino acid composition & high digestibility required to meet human requirements thus the ability of dietary proteins to fulfil this role varies widely.

Requirements vary with age, physiological state, state of health : Important consideration – all amino acids have imp physiological roles in synthesis and functioning of muscles & organs, as well as enzymes, hormones, immune system

Besides the INDAA- some become conditionally indispensable eg arginine, cysteine, glutamine, glycine, proline and tyrosine, can become conditionally indispensable, e.g., for premature neonates

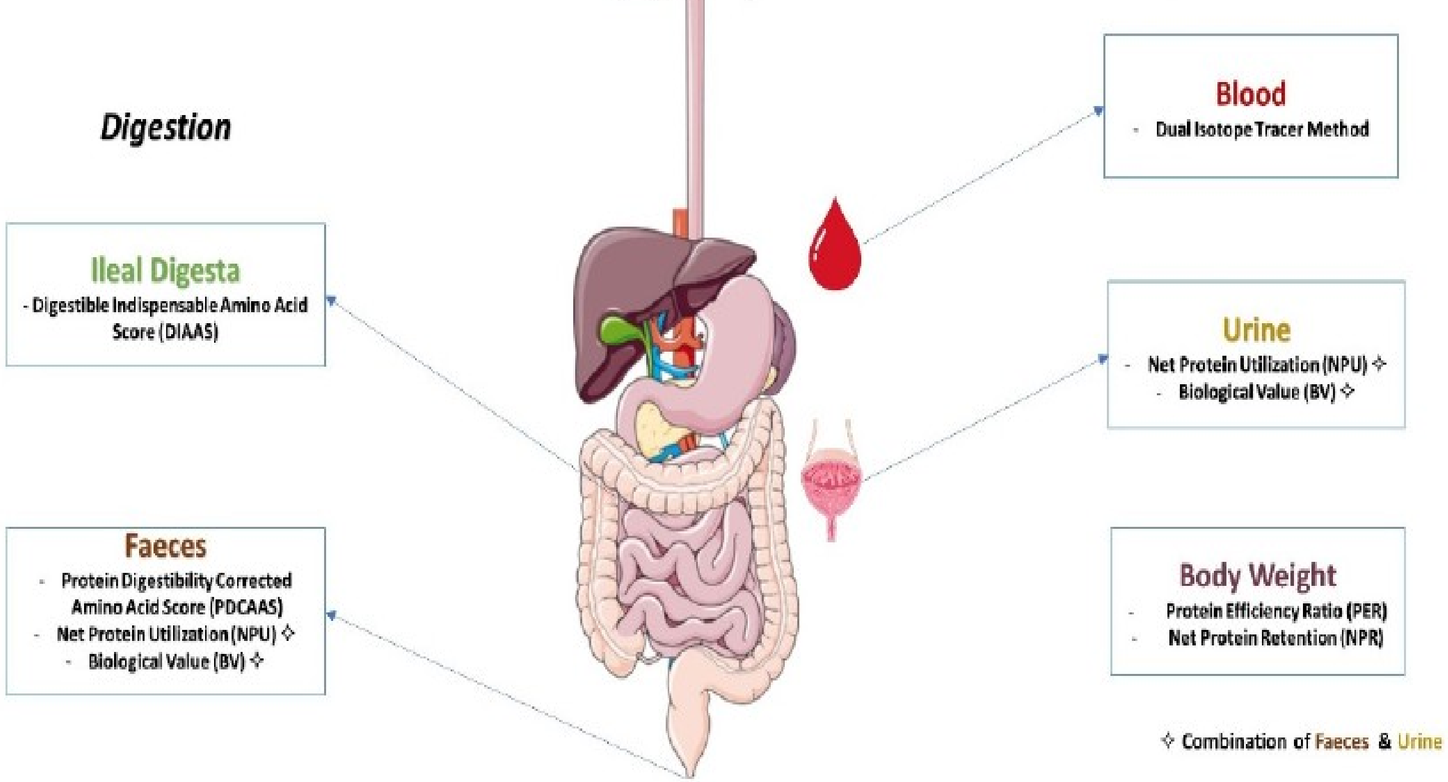
Such quality is influenced by the availability of amino acids, which depends on various factors like protein origin, previous processing treatments, and interactions with other food components

FIGURE 2.
Framework depicting short- and long-term potential protein quality related health outcomes. This indicates the need to look beyond physiological and metabolic responses in assessing health effects



Reference protein digestibility – 100%

Protein Quality Measurement



Method	Measurement Principle
Protein quality methods	
Protein efficiency ratio (PER)	Ratio of weight gain and protein consumed by test group over control (preferred reference protein: casein)
Net protein ratio (or net protein retention) (NPR)	Difference in weight gain between a test protein group and protein-free diet group per gram of protei consumed by the test protein group.
Protein digestibility corrected amino acid score (PDCAAS)	Ratio of IAA_{lim} in test protein compared to referenc protein corrected for faecal protein digestibility
Digestible indispensable amino acid score (DIAAS)	Ratio of IAA_{lim} in test protein compared to referenc protein corrected for ileal digestibility of IAA_{lim}
Protein digestibility methods	
True Digestibility (TD)	Percentage of nitrogen observed from protein (food) consumed in the GI tract
Biological value (BV)	Retained nitrogen over total nitrogen intake, with corrections for faecal and urinary losses.
Net protein utilization (NPU)	Retained nitrogen over total nitrogen intake, with corrections for faecal and urinary losses.
Dual isotope tracer method	Compares AA in circular system from intrinsically labelled test protein consumed together with a reference protein with known digestibility labelled differently

Figure 3. Overview of site of measurement for different in vivo protein quality measurement methods.

Adhikari, S.; Schop, M.; de Boer, I.J.M.; Huppertz, T. Protein Quality in Perspective: A Review of Protein Quality Metrics and Their Applications. Nutrients 2022, 14, 947.

Figure 1. Protein quality evaluation through time. Protein efficiency ratio, PER; net protein utilization, NPU; biological value, BV; protein digestibility-corrected amino acid score, PDCAAS;

Joint FAO/WHO Expert Consultation

Tasks:

- Review present knowledge related to protein quality evaluation
- Discuss various techniques used for evaluation
- Specifically evaluate the method recommended by the Codex Committee on Vegetable Proteins (CCVP) : amino acid score corrected for digestibility

For sometime amino acid score used—Some but not all proteins can be evaluated due to poor digestibility and/or availability

Methods currently used established when extensive information unavailable on human amino acid requirements

Most methods used a rat assay– misleading to some extent as rat requires more sulfur amino acids than humans – also histidine and BCAA

Limitations of different methods

US FDA currently uses the Protein Digestibility-Corrected Amino Acid Score (PDCAAS) to measure protein quality in most foods , Canadian government utilizes the PER

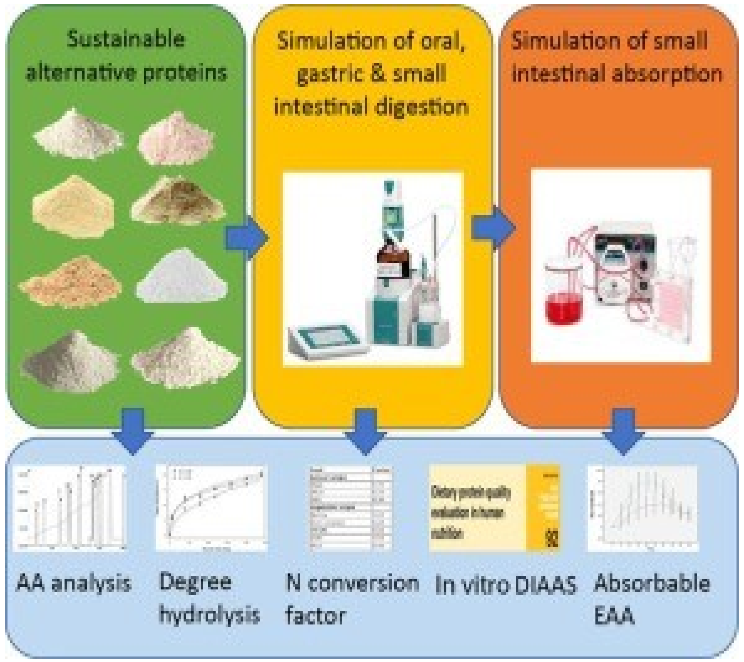
Does not properly credit maintenance requirements (based on rat growth studies)

A protein does not support growth – PER=0 but may be adequate for maintenance

Values not proportional to quality: eg PER of 2 does not make the protein 2X as good as one with a PER of 1

Unsuitable to calculate utilizable protein- eg in protein rating- protein in a reasonable daily intake – mass x PERAnimal sources of protein (i.e., meat, seafood, and dairy) tend to rank higher than plant proteinsources:

Why??? high digestibility, a distribution of the 9 EAA considered perfectly aligned with human requirements,



Food matrix of plant proteins partially impairs digestion and the EAA distribution can be proportionally low, relative to dietary requirements, in one or more.

For example, grains tend to be proportionally low in lysine, whereas legumes are proportionally low in methionine

FAO recommendations: ***Boutrif (2022) Recent developments in protein quality evaluation***

What about digestibility?

- Studies needed to compare protein digestibility values of humans and rats from identical food products
- Extensive evaluation indicates rat balance method most practical for predicting protein digestibility by humans - particularly when human balance studies difficult
- Recommended that amino acid scores be corrected only for true digestibility of protein
- For new, novel products/processes need to determine digestibility values
- Need to establish data base for raw& processed products
- Established digestibility values of well-defined foods may be taken from a published database for use in amino acid scoring procedure

Protein	Amino Acid Score	True Protein Digestibility (%)*	PDCAAS
Pea (yellow, split)	0.73	87.9	0.64
Pea (green, split)	0.59	85.2	0.50
Lentil (green, whole)	0.71	87.9	0.63
Lentil (red, split)	0.59	90.6	0.54
Chickpeas (Kabuli)	0.61	85.0	0.52
Pinto Beans	0.77	76.2	0.59
Kidney Beans	0.70	78.6	0.55
Black Beans	0.76	70.0	0.53
Navy Beans	0.83	80.0	0.67
Wheat Flour	0.47	92.3	0.43
Rice Flour	0.54	92.0	0.50
Soy Flour (50% protein)	0.92	83.5	0.77
Pea protein Isolate (82% protein)	0.54	97.1	0.53
Pea Protein Concentrate (50% protein)	0.58	92.6	0.54
Soy Protein Isolate (93% protein)	0.87**	96.0	0.84
Casein	1.04	96.6	1.00

*True fecal nitrogen digestibility
**Other sources (e.g., Hughes, G.J., *et al.*, http://dx.doi.org/10.1021/jf203220v, 2011) have calculated a PDCAAS for soy protein isolate of 1.00.

Credit: "Protein quality of cooked pulses," Pulse Canada (https://tinyurl.com/pulsecanada-cooked)

The case for plant proteins and complementation

- When a variety of plant protein sources consumed in sufficient quantities, (would be true of almost any dietary pattern) – to include appropriate variety and quantity to meet other nutrient requirements, needs for essential amino acids can be met without any animal protein intake.

ANIMAL PROTEIN VERSUS PLANT PROTEIN

ANIMAL PROTEIN	PLANT PROTEIN
Sources such as meat, fish, poultry, eggs, and dairy, which are similar to the protein found in the body	The sources of vegetables, whole grains, legumes, seeds, and nuts
A complete protein, containing all essential amino acids	Incomplete protein, providing only several essential amino acids to the diet
90% Absorbable	60-70% Absorbable
85% Digestible	95-100% Digestible
High in calories	Low in calories
Rich in saturated fat, sodium, calcium, zinc, phosphate, and vitamin B12	Rich in unsaturated fat, fiber, potassium, magnesium, and folate
Contains heme iron, which is highly bioavailable	Contain non-heme iron
Low in antioxidants	High in antioxidants
Contains a higher amount of uremic toxins and harbors proteolytic bacteria	Contains a low amount of uremic toxins and harbors saccharolytic bacteria
Has negative health effects	Has positive health effects

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Recommendations

- Previously used reference patterns egg or milk proteins
- Substitute -- a provisional pattern of amino acid requirements for the egg protein standard
- Hypothetical based on pattern of amino acid requirements – standard for comparison

- Critical for success is ability to obtain precise measurements of amino acid content in protein sources

- Improve on accuracy of scoring methods --- chemically determined contents may need to be corrected for digestibility or biological availability
- Currently recommended for use:

  * human milk amino acid composition for infants under one year of age

  *Amino acid scoring patterns recommended for children of preschool age- FAO/WHO/UNU (1985)
- Deemed as temporary until further research confirms adequacy or necessitates a revision

Issues/Challenges in Quality Evaluation

The DIAAS determines amino acid digestibility, at the end of the small intestine, providing a more accurate measure of the amounts of amino acids absorbed by the body and the protein contribution to human amino acid and nitrogen requirements

However, as research continued to evolve in assessing protein's role in optimal health at higher intakes, there was also a need to continue to explore implications for protein quality assessment

Use of metabolomics approaches? relating complex metabolite profiles from plasma and urine samples to protein and amino acid true ileal digestibility and availability oer a promising perspective for the evaluation of dietary protein quality in humans (FAO, 2013).

A second important issue in quality evaluation relates to the bioavailability or digestibility of a protein or the capacity to provide metabolically available nitrogen and amino acids to tissues and organs. A protein can be predicted as being of good quality based on its amino acid score, but in practice may be of poor quality because it is poorly digested and/or absorbed.

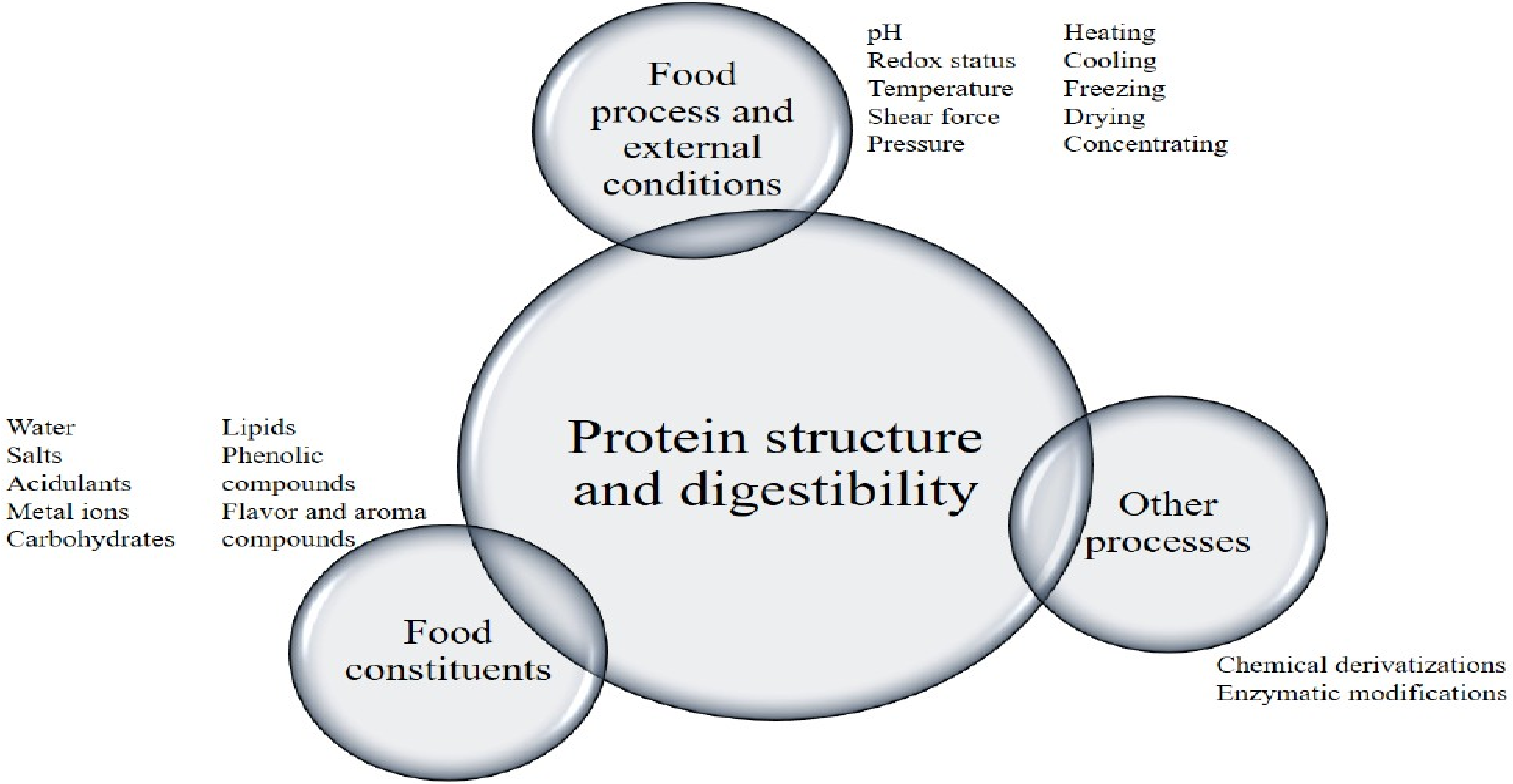


Figure 2. Factors which determine the structure and digestibility of food proteins. Adapted from Yada (2018

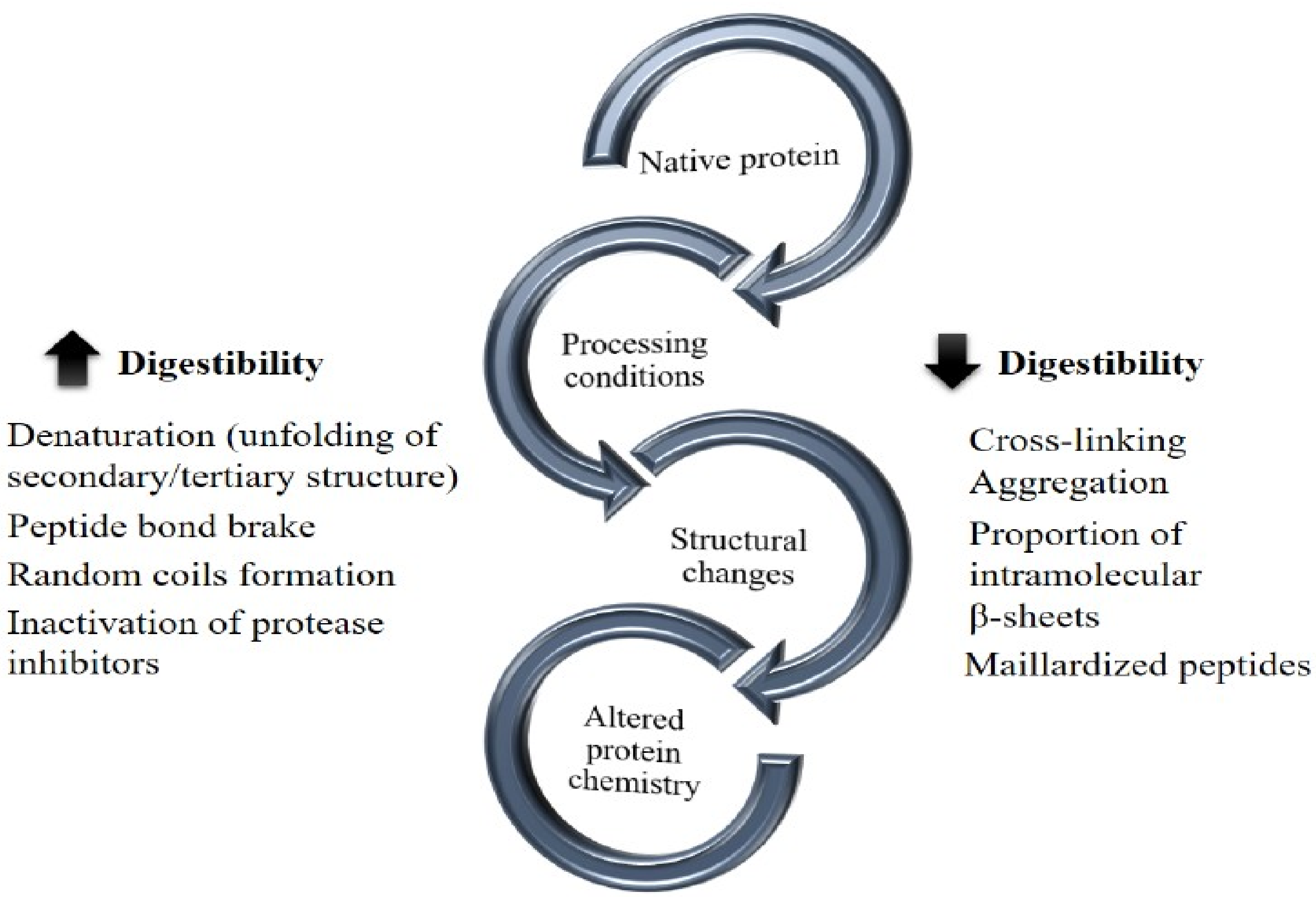


Figure 3. Structural changes that modify protein digestibility.

Table 1. Factors which impair enzymatic protein digestion.

Naturally occurring limiting structures	Animals	Scleroproteins such as collagen, elastin, keratin, and silk fibroin that form supporting structures in the body and are resistant to digestion due to their unusual structures.
	Plants	Plant proteins have lower digestibility due to their relative insolubility, intracellular organization in discrete protein bodies, and protective covering of the seed by the seed coat. They usually require some processing to improve the protein digestibility.
Processing treatments	Heat-treatment	Enhances polymerization and changes in secondary structure which decreases enzymatic digestibility of sorghum proteins.
	Maillard reaction	Causes a decrease of protein nutritional quality due to a condensation reaction between the carbonyl group of a reducing carbohydrate and the free amino groups of a protein, which originates Maillardized peptides that cannot be absorbed by the gut.
	Irradiation	Reduces protein digestibility due to cross-linking and to the formation of Maillard products, which inhibit enzymatic protein digestion.
Anti-nutritional factors	Tannins	They have been linked to weight gain reduction due to their inhibition of digestion of dietary proteins. Apparently, they reduce feed digestibility by the formation of tannin-nutrient complexes.
	Protease inhibitors	Inhibit the activity of trypsin and chymotrypsin, thus preventing protein digestion.
Changes in chemical structures	Disulfide bonds	They stabilize the protein structure making it more resistant to proteolytic degradation.
	Cross-linking	Lowers the digestibility of food because the cross-linked, aggregated protein is less accessible to digestive enzymes.
	Oxidation	Impairs protein function, leading to an increase of protein hydrophobicity, which results in the formation of toxic aggregates. Diminishes the sensory and nutritional protein quality due lysin and sulfur amino acids loss.

Adapted from Becker and Yu (2013)

Table 2. Selected food processing methods effect on selected proteins digestibility.

Food product	**Effect on protein digestibility**	**Reference**
Vegetable feed ingredients	During thermal processing, proteins reacted with reducing sugars to produce Maillard products that decreased the digestibility of the protein	Salazar-Villanea *et al*. (2016)
Lentil and faba bean concentrates	High pressure processing produced greater gastric digestibility	Hall and Moraru (2021)
Faba bean isolate	Ultrasonication treatment decreased protein digestibility	Martínez-Velasco *et al*. (2018)
Soybean milk	Microwave treatment increased soy proteins digestibility	Vanga *et al*. (2020b)
Shrimp	Microwave treatment (125 °C, 15 min) decreased the allergenicity of tropomyosin and *in vitro* digestibility	Dong *et al*. (2021)
Beef	Freezing-then-aged treatment (FA) was applied and compared to an only aged control. Post *in vitro* digestion (14 days aged) showed that FA had enhanced protein digestibility	Lee *et al*. (2021)
Muscle foods	Ultrasound can induce denaturation and affect de unfolding-refolding of proteins, affecting the diffusion of proteases into the protein matrix and their accessibility to cleavage sites, increasing digestibility	Bhat *et al*. (2021b)
Milk protein	Milk proteins exposed to various heat treatments (temperature, time) induced changes on the digestibility of the protein, which could be used for tuning the gastric coagulation behavior of milk proteins.	Li *et al*. (2021)
Buffalo and cow milk	Microfluidization improved lactose and protein stability and in vivo Wistar rat digestibility.	Kumar *et al*. (2019)
Liquid whole eggs	Showed no *in vitro* digestibility differences when thermally treated at 60°C for 10 min with untreated control, but digestibility was improved when treated at 80 °C for 10 min.	Bhat *et al*. (2021a)
Egg white proteins	Thermally treated at 65°C for 30 min, exhibited higher digestibility than when treated at 56°c for 32 min or !00 °C for 5 min. Applying HPP in the range of 400?700 MPa led to the formation of aggregates stabilized mainly by SS bonds. Increasing pressures increased the formation of protein aggregates, which were more prone to enzymatic hydrolysis.	Farjami *et al*. (2021)

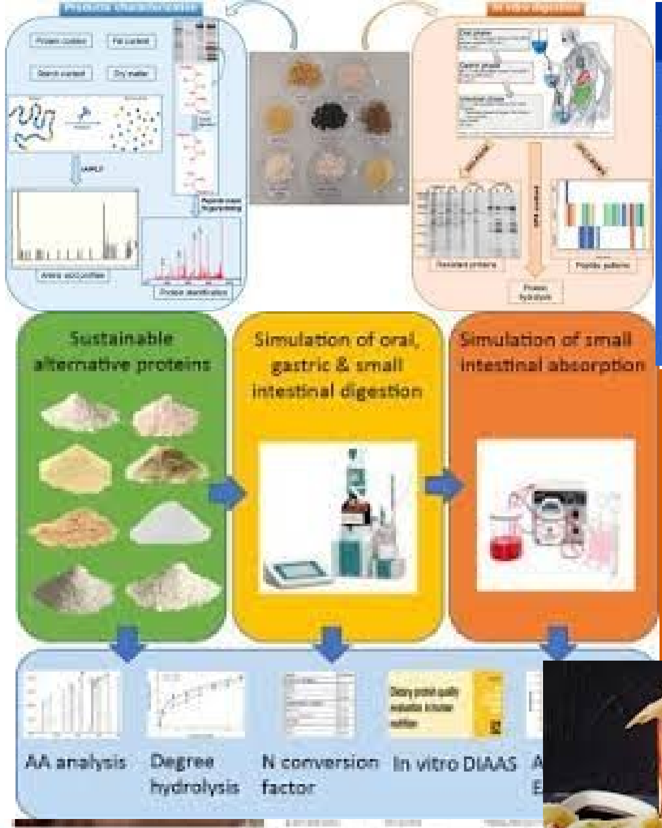
Table 1: Lysine and Methionine content and percent of requirement obtained from select cereals, pulses and cereal-pulse combinations

	Lysine (mg/g of protein)	Lysine % requirement	Methionine (mg/ g of protein)	Methionine % requirement
Rice	37.0	82.2	26.0	162.5
Wheat flour	24.2	53.8	17.7	110.6
Red gram dal	61.6	136.9	8.7	54.4
Green gram dal	60.9	135.3	10.5	65.6
Bengal gram dal	60.6	134.7	11.2	70.0
Khichadi (Rice + Tur dal*)	50.8	113.0	16.3	101.7
Khichadi (Rice + Moong dal*)	51.0	113.3	16.9	105.7
Wheat roti + Dal*	42.6	94.6	13.3	83.0

Table 2: Lysine and Methionine content and percent of requirement provided by select vegetable-pulse combinations

	Lysine (mg/g of protein)	Lysine % requirement	Methionine (mg/g of protein)	Methionine % requirement
Cabbage	31.2	69.3	10.6	66.3
Chana Dal	60.9	135.3	10.5	65.6
Cabbage chana dal (Veg:Pulse::100:20)	53.5	119.0	11.1	69.1
Bottle gourd	48.1	106.9	9.4	58.8
Chana Dal	60.9	135.3	10.5	65.6
Chana Dal dudhi (Veg:Pulse::75:25)	59.7	132.8	11.1	69.2
Spinach	2186.9	4859.8	621.5	3884.3
Red gram dal	61.6	136.9	8.7	54.4
Tur dal palak (Veg:Pulse::20:25)	217.0	482.3	53.5	334.4
Colocasia Leaves	17.9	39.8	44.3	276.9
Chana Dal	60.9	135.3	10.5	65.6
Patra (Chana Dal Flour + Colocasia Leaves) (Veg:Pulse::25:70)	58.3	129.6	13.0	81.1
Cauliflower	41.3	91.8	10.1	63.1
Chana Dal	60.9	135.3	10.5	65.6
Cauliflower Bhajiya (Veg:Pulse::50:20)	56.7	126.1	11.0	68.6
French Beans	47.7	106.0	8.3	51.9
Red gram dal	61.6	136.9	8.7	54.4
Green Bean ParuppuUsili (French Beans + Tur Dal) (Veg:Pulse::75:20)	57.4	127.6	8.6	53.6

All values are based on the content given in the IFCT 2017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Simulation of oral, gastric & small intestinal digestion
Simulation of small intestinal absorption
AA analysis
Degree hydrolysis
N conversion factor
In vitro DIAAS


How to Measure Protein Digestion with Stable Isotopes
Phase 1
Phase 2


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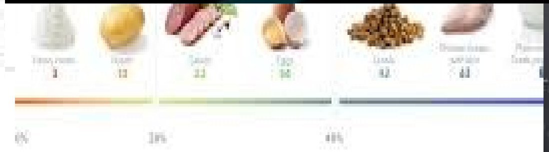

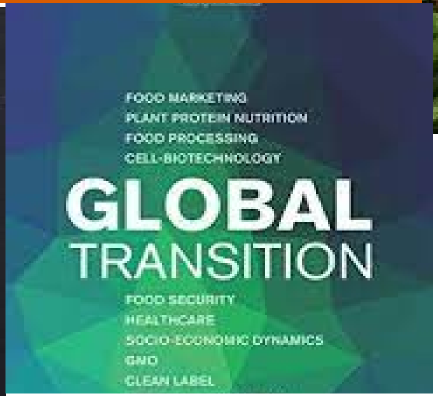
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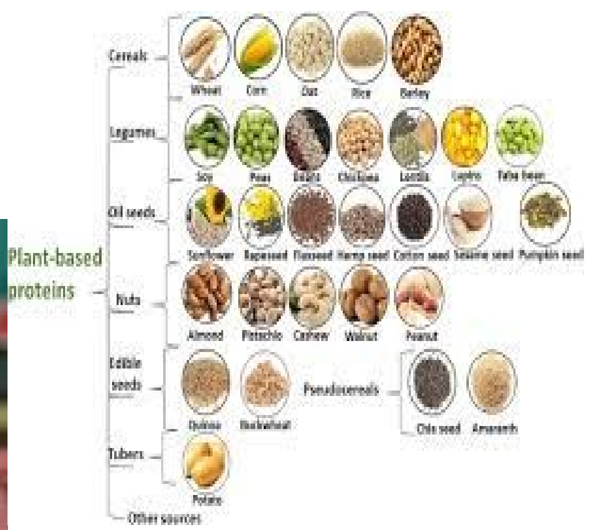
Plant-based proteins
Cereals
Legumes
Oil seeds
Nuts
Edible seeds
Tubers
Other sources
Pseudocereals


Curcumin
SPI
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Enhanced stability and bioaccessibility of curcumin
Improved protein digestibility