Methods of protein quality and quantity analysis for nutritional labeling and to verify protein claims

Sneh D. Bhandari

Independent Consultant

Crete, IL

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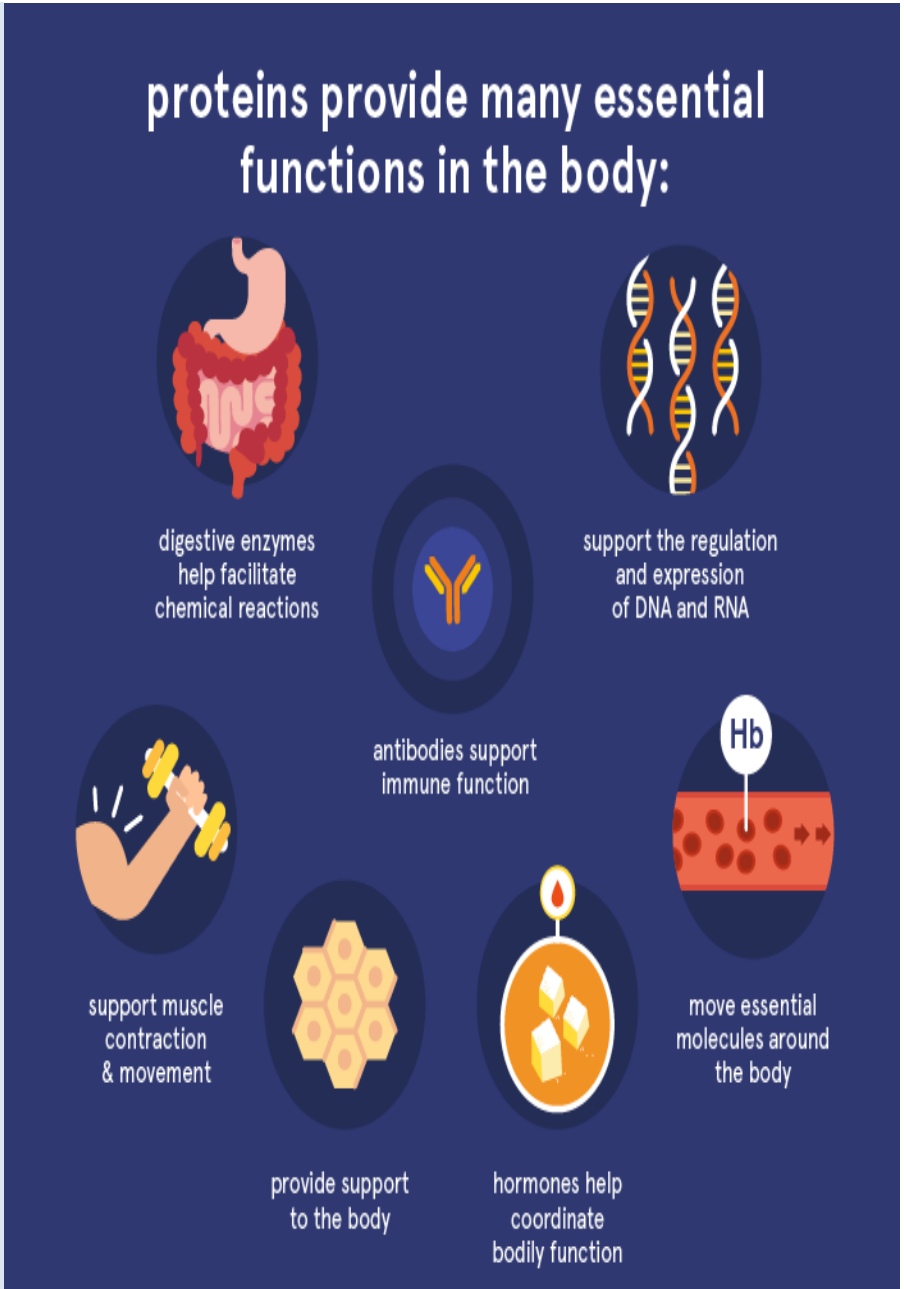
AGENDA

- Background - Protein/AA
- Health Effects of proteins in diet
- Protein & Amino Acid analysis
- Protein Quality
- Protein Quality- PDCAAS & DIAAS
- Protein Nutrition Labeling & Claims

Main Functions of Proteins in Body

- Structural components
- Growth, development & repair
- Physiological Metabolic , Immune & Muscle functions
- Reproduction
- Energy production
- O_2 & nutrient transport
- Blood clotting,
- Fluid balance,
- Vision,
- Hormones, antibodies, enzyme



Amino Acids – Protein quality definer

Indispensable Amino acid (IAA)	Conditional Dispensable AA	Dispensable AA
Leucine	Arginine	Alanine
Isoleucine	Cysteine	Aspartic Acid
Valine	Glutamine	Aspargine
Lysine	Glycine	Glutamic Acid
Threonine	Proline	Serine
Methionine	Tyrosine	
Tryptophan		
Phenylalanine		
Histidine		

Special Functions of Branched Chain Amino Acids

EAA – Leu, Ileu & Val : Branched chain AA

BCAAs promotes muscle protein synthesis in athletes

Recovery of muscle damage & fatigue due to exercise

Stimulate muscle protein synthesis in older individuals also

Source: Fujita and Volpi (2006). J Nutr. 36: 277S; Holeček (2018) Nutrition & Metabolism 15:33

Features of animal and plant protein sources

Features	Animal Protein	Plant Proteins
Health Risk Factors	Substitution of Red processed meat by plant proteins --> lower CVD mortality	
Saturated Fats & Cholesterol	High	Low & None
High Sodium	Processed meats	Generally not an issue
IAA	Complete proteins	Not of enough all IAA
Dietary Fiber & Phytochemicals	Plant foods rich in dietary fiber, phytonutrients	
Micronutrients	Most vit (- Vit D) & minerals high in plant foods; Vit. A, D, B6, B12 + certain minerals in some AF	
Antibiotic resistance	Possibility of animal-to-human transmission of antibiotic resistance AF	

Source: Source: Richter et.al. (2015) Adv. Nutr. 6:712; Marshal & Levy (2011) Clin. Microbiol. Rev. 24: 718

Health impact of plant protein

Too much red & processed meat associated with CVD Mortality

Plant-based diets leads to lower risks of CVD, other diseases & mortality

Plant based foods environmentally more sustainable

*Source: Song et.al. (2016) AMA Intern Med. 176:1453; Melina et al (2016) *J Acad Nutr Diet*. 116:1970

Consumer demand of new protein foods with good quality & quantity

Consumers like plant food - due to their health benefits & AF's global warming concerns.

Food industry developing new plant protein foods

Determn of protein quantity & quality - important in protein nutrition.

Protein = Nitrogn (g) by Kjeldahl/ Combustn X Prot. CF. NPN correction if helps i.e. sea food, insects etc.

Protein quality evaluation requires AA (IAA) analysis of & protein digestibilit y in food.

AOAC & other Kjeldahl methods of protein estimation

Matrix	Factor#	AOAC Method	Matrix	Factor#	AOAC Method
Flour	5.70	920.87	Dairy	6.38	930.29
Grain (- wheat)	6.25	979.09	Milk	6.38	991.20
Wheat	5.70	979.09	Ice-cream	6.38	930.33
Bread	5.70	950.36	Frozen deserts,	6.38	930.33
Maccaroni products	5.70	AOAC 930.25	Milk Chocolate	6.38	939.02
Baked Products	5.70	935.39	Almond	5.18	950.48
Soybean	5.71 (FAO)	AOCS Bc 4-41	Peanuts Brazil Nuts	5.46	950.48
Pulses	6.25	ISO 20483:2006	Other tree Nuts Coconut	5.30	950.48
Oil seed byproducts	6.25	AOCS Ba 4d-90	Fruit	6.25	920.152
Meat	6.25	981.10	Beer	6.25	920.53

N to protein calculation by multiplication factor

Amino Acid Analysis of Proteins: Acid hydrolysis & HPLC

Protein Hydrolyzed to release peptide bound AA in 6M HCl for 18-24 h @110°C in vacuo or under N2

- **Released AA analyzed commonly by HPLC**

SH-AA destroyed: Protect with performic oxidation prior to hydrolysis (AOAC 985.28)

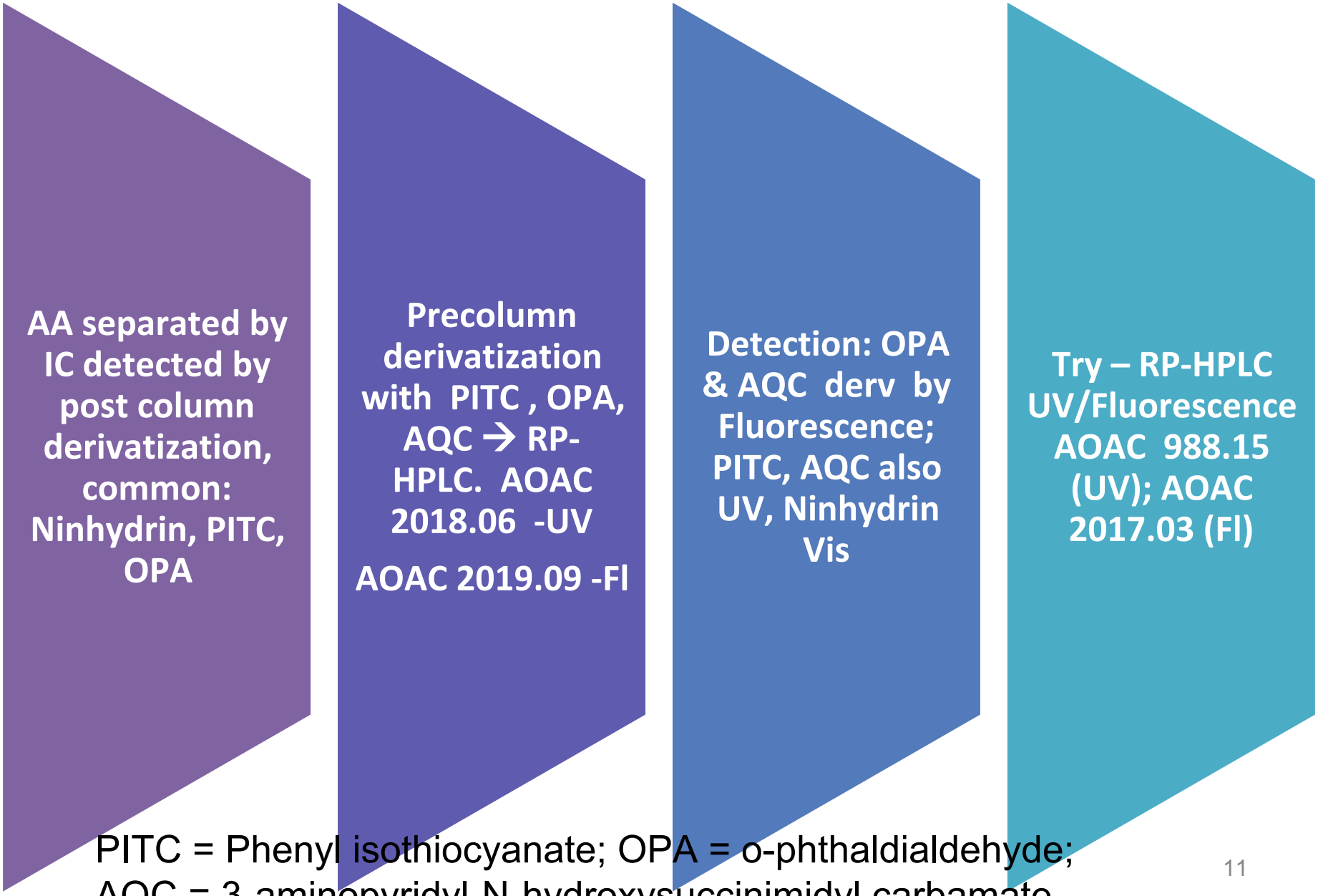
Try destroyed: do Alkaline (AOAC 988.15) or Enzymatic hydrolysis (AOAC 2017.03)

Asn → Asp: Analyze sum of Asn + Asp.

Gln → Glu: Analyze sum of Gln + Glu

Other AA: Phenol ; thioglycolic acid for SH-AA

Amino Acid Analysis commonly performed by HPLC



PITC = Phenyl isothiocyanate; OPA = o-phthaldialdehyde;
AQC = 3-aminopyridyl-N-hydroxysuccinimidyl carbamate

Protein Quality is important in protein nutrition

Nutritional labels from different foods - % DV for protein not often listed – one reason, needs quality check to qualify

Method of Protein Quality Evaluation for foods > 1 year ages: Protein Digestibility Corrected Amino Acid Score (PDCAAS)

PDCAAS is AA score calculation = Relative IAA amount corrected for protein digestibility (PD)

Protein (g/serving) mandatory for labeling ; %DV (Voluntary) , reqird for food of 1- 3 years age or protein claims & needs gm x PDCCAS

Protein Efficiency Ratio (PER) is quality tool for food ≤1 year. Canada's Protein Rating is PER based. PDCAAS allowed if no PER

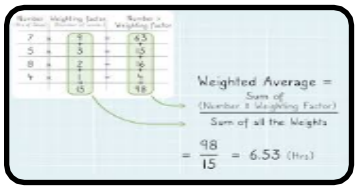
PDCASS and Amino Acid Score Estimation

Method/Action	Specification
Estimate amount of protein & IAA	Use N to protein conversion factor specified in applicable AOAC methods
Compare conc. of each IAA (Meth + Cys & Phe +Tyr) mg/g protein against reference pattern	IAA in least amount vs FAO/WHO 1991 reference pattern (2-5 yr child), limiting amino acid
Relative least amount of IAA in protein	Amino Acid Score (AS)
PDCAAS = AS x Protein Digestibility	Values >1 truncated to 1. PD commonly estimated by Rat fecal N balance method

True Digestibility Value of proteins/amino acids

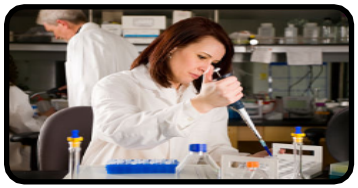
Rat fecal-balance (% of N intake retained) = Ingested N – (Fecal N - endogenous N loss).

PD values (Human or Rat) of foods available (literature); if not = Determine



PD of a product can be calculated by a weighted average of ingredient's PD

Pig Ileal Digestibility of AA: FAO suggested (2013) for DIAAS*. Data base developing.



In vitro assays are available, no particular method recommended by FAO/WHO. Megazyme offers a kit for in vitro assay

*DIAAS = Digestible Indipensable Amino Acid Score

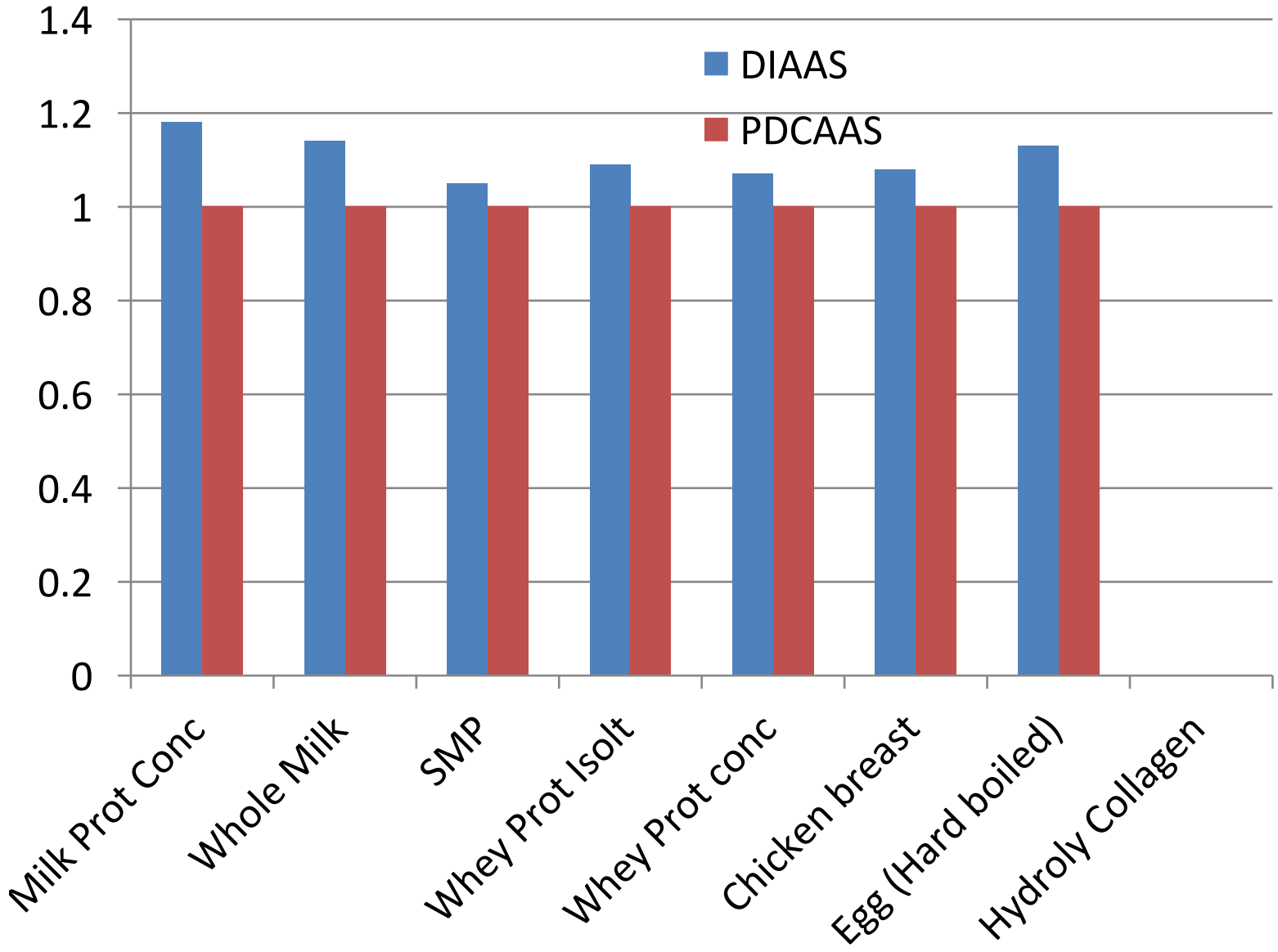
PDCAAS Calculation Example

EAA	EAA in Sample (g/100g)	EAA in Sample mg/g protein	EAA Reference Pattern mg/g protein	EAA in sample/Ref Pattern
Isoleucine	1.17	46.1	28	1.65
Leucine	1.96	77.2	66	1.17
Lysine	1.64	64.6	58	1.11
Total SAA	0.46	18.1	25	0.72
Total AAA	1.74	68.6	63	1.09
Threonine	0.85	33.5	34	0.99
Tryptophan	0.175	6.9	11	0.63
Valine	1.22	48.1	35	1.37
Met	0.19	**Amino Acid Score**		**0.63**
Cysteine	0.27	*Protein Digestibility*		*0.86*
Phe	1.13	PDCAAS = AA Score x PD		
Tyr	0.61	***PDCAAS = 0.63 x 0.86***		***0.54***
Protein (g/100g)	25.38			

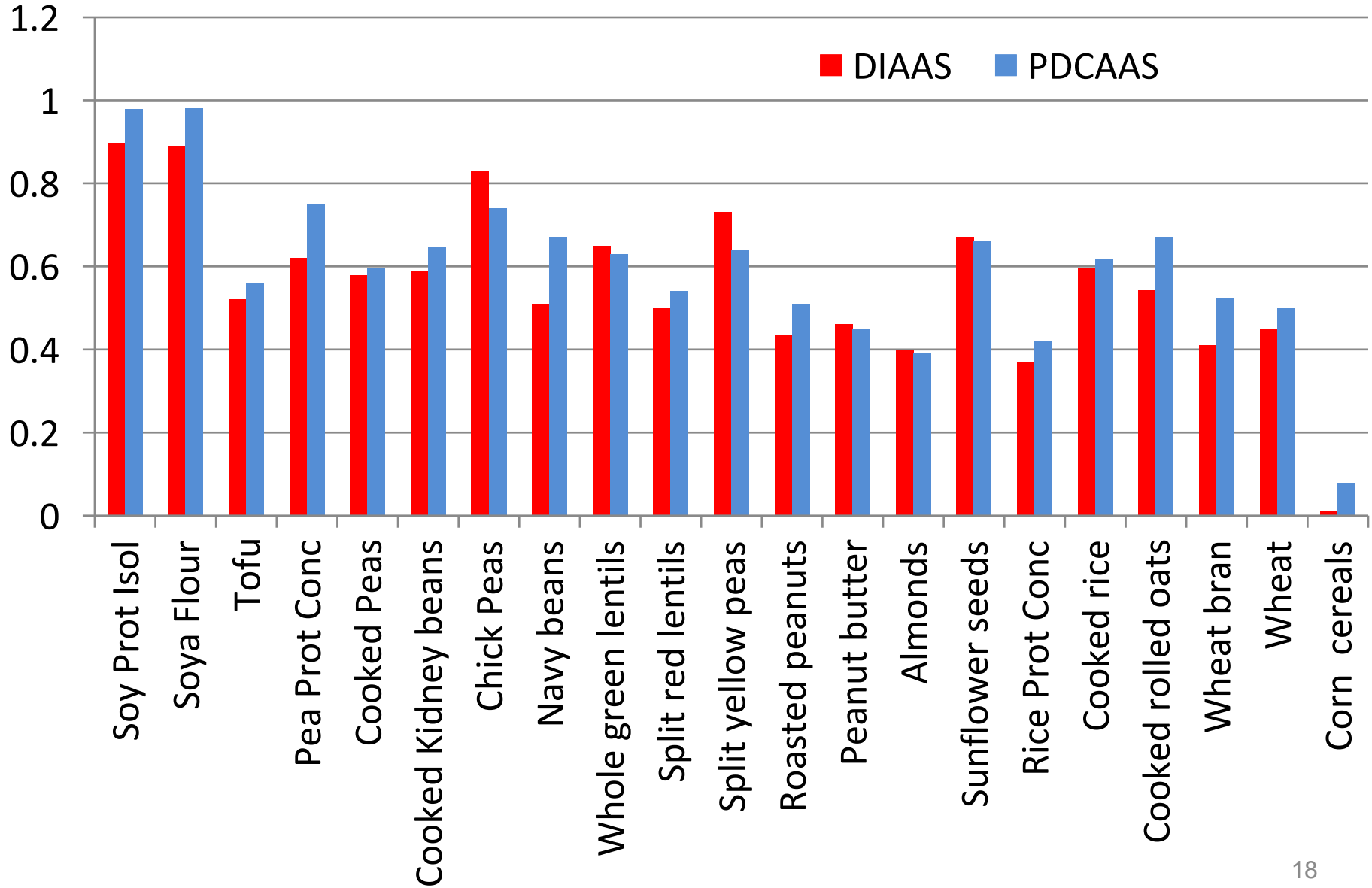
Protein Quality – PDCAAS Issues addressed by DIAAS

Concerns about PDCAAS	FAO recommended DIAAS (2013) to address PDAAS concerns
PD estimation based on rat fecal N estimate	Sampling done from Pig ileum
PD less accurate	Estimated digestibility of each IAA
PDCAAS >1 truncated to 1	Reports values >1, no underestimation
AA requirements of 3-5 years (Except infant)	3 patterns - Infants (6 months), Children (6 months to 3 years) & Others > 3 years.
FDA declined in 2016 to replace PDCAAS by DIAAS	Insufficient IAA digestibility data of different foods; Not convinced about suggested AA requirement patterns

PDCAAS and DIAAS of common animal foods



PDCAAS and DIAAS of common plant foods



Protein Nutrition Labeling

#	FDA Guidelines for Protein Labeling
1	N to protein conversion factor of 6.25 or in AOAC method of protein analysis.
2	Report protein in grams/serving to the nearest gram
3	Amount /Serving < 0.5 gram, may be labeled as 0.
4	Voluntary declaration of % DV for protein on label
5	% DV labeling required if Protein Claim made; Food for infants/children ≤3 yrs.
6	New Nutritional Labeling regulation of 2016 – No changes in protein declaration
7	Corrected protein g/serving = g protein/serv X PDCAAS
8	% DV= Corrected of protein (g) per serving/ RDI or DRV X 100 (to nearest 1% increment).
9	DRV = 50 g(adult). RDI = 11 g (≤ 1 yr)

Labeling Protein

Pork Rinds Label –"Not a significant source of protein"

Original Label

Nutrition Facts

Serving Size 2/3 cup (55g)
Servings Per Container About 8

Amount Per Serving

Calories 230	Calories from Fat 72
	% Daily Value*
Total Fat 8g	**12%**
Saturated Fat 1g	**5%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 160mg	**7%**
Total Carbohydrate 37g	**12%**
Dietary Fiber 4g	**16%**
Sugars 1g	
Protein 3g	
Vitamin A	10%
Vitamin C	8%
Calcium	20%
Iron	45%

* Percent Daily Values are based on a 2,000 calorie diet. Your daily value may be higher or lower depending on your calorie needs.

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

New Label

Nutrition Facts

8 servings per container
Serving size **2/3 cup (55g)**

Amount per serving
Calories **230**

	% Daily Value*
Total Fat 8g	**10%**
Saturated Fat 1g	**5%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 160mg	**7%**
Total Carbohydrate 37g	**13%**
Dietary Fiber 4g	**14%**
Total Sugars 12g	
Includes 10g Added Sugars	**20%**
Protein 3g	
Vitamin D 2mcg	10%
Calcium 260mg	20%
Iron 8mg	45%
Potassium 235mg	6%

* The % Daily Value (DV) tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

Protein Quality and Nutrition Labeling -

Age group	PDCAAS Score	Nutr. Label column "Percent Daily Value"
4 years to adult	<20	"not a significant source of protein,"
>1 years to < 4 yr	<40	
4 years to adult	≥20	nearest whole percent placed, optional if no protein claim made
Children 1 through 3 years	≥40	nearest whole percent must be placed
infants through 12 months	Relative PER <40% of reference *	"Not a significant source of protein"
infants through 12 months	Relative PER ≥ 40% of reference*	nearest whole percent must be placed

Source: e-CFR data is current as of April 1, 2019, Title 21 → Chapter I → Subchapter B → Part 101 → Subpart A → §101.9; * reference = Standard Casein

Protein Nutrition Labeling – Common Nutritional claims

Claim	Protein (% of DRV) per RACC*
High, Rich In, or Excellent Source Of - Protein	≥ 20%
Good Source, Contains, or Provides - Protein	10-19%
More, Fortified, Enriched, Added, Extra, or Plus	≥10% than an appropriate reference food

*RACC = reference amount customarily consumed

Protein spiking in Sports Nutrition Industry

Protein in food for nutritional labeling often determined by its N estimation.

Protein spiking is addition of NPN to a product to inflate its label value & possibly make a claim

The sports nutrition industry has seen some instances of the practice of “protein spiking“.

In these cases NPN in the supplement/food is not adjusted in protein estimation.

Companies have been sued for selling supplements with NPN inflated protein label values.

Hi-Tech Pharmaceuticals Won an Appeal at 11th Circuit against AllMax for Protein Spiking (2018).

Review

A trend of high demand of healthy (plant) quality protein foods.

Protein & IAA analyzed for nutr. labeling, Protein often by N analysis . Protein AA released by hydrolysis → analyzed by HPLC.

Nutritional quality of protein evaluated by PDCAAS based on AA score & PD. Used for corrected amount of protein/SS.

DIAAS a quality tool proposed to address PDCAAS shortfalls but it has not been adopted yet in the lack of data base & consensus.

Protein (g/ss) declared in nutr. label ; %DV (Voluntary) , must for food of 1- 3 yrs; protein claims; PDCAAS adjusted protein needed.

PER estimates protein quality of food for ≤12 mnths. Canada's food Protein Rating - PER based. PDCAAS used in the lack of PER.

THANK YOU