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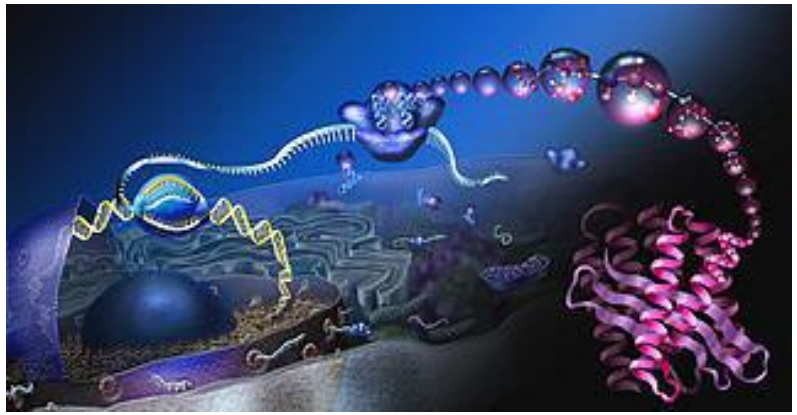
NC STATE UNIVERSITY | BEZOS EARTH FUND

Sustainable Food Proteins Course
Minneapolis – October, 2025

NC FOOD INNOVATION LAB

BRIDGE2FOOD

Lecture #2
Protein Functionality



Lecture Topics:

- Protein chemistry review
- Biomanufacturing of plant-based protein products
- Physical functionality
- Physiological functionality
- Protein concentrates and isolates

Ref.: https://commons.wikimedia.org/wiki/File%253AHow_proteins_are_made_NSF.jpg

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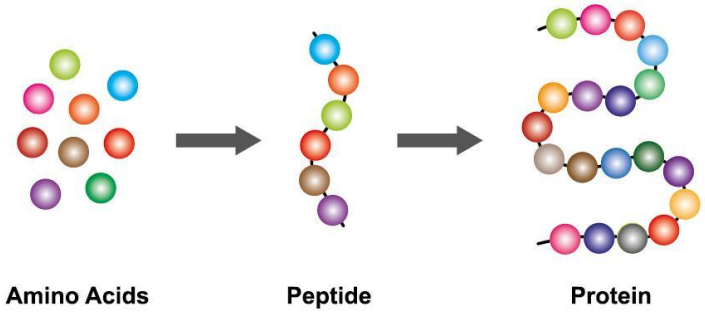
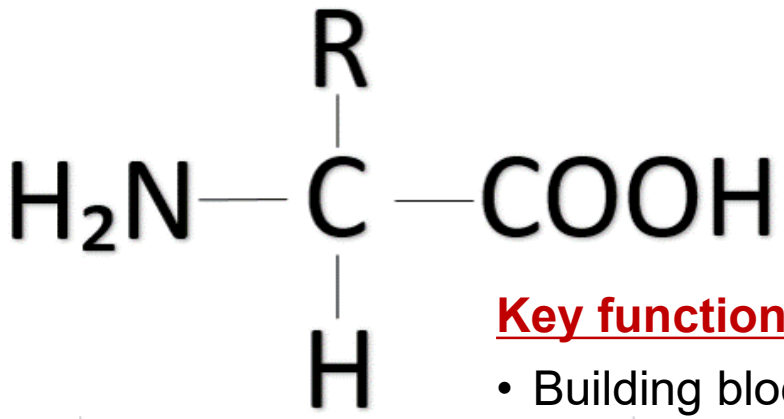
Amino Acid Basics: The Building Blocks of Proteins Basics



Amino acid: organic molecule made up of a basic amino group (NH2), an acidic carboxyl group (-COOH), and an organic R group (or side chain) unique to each amino acid



Key functions of proteins:

- Building blocks of cells, tissues, and organs – role in growth, development, and repair
- Regulation of hormones and enzymes
- Nutrient transport
- Immune system support
- Providing energy in certain situations

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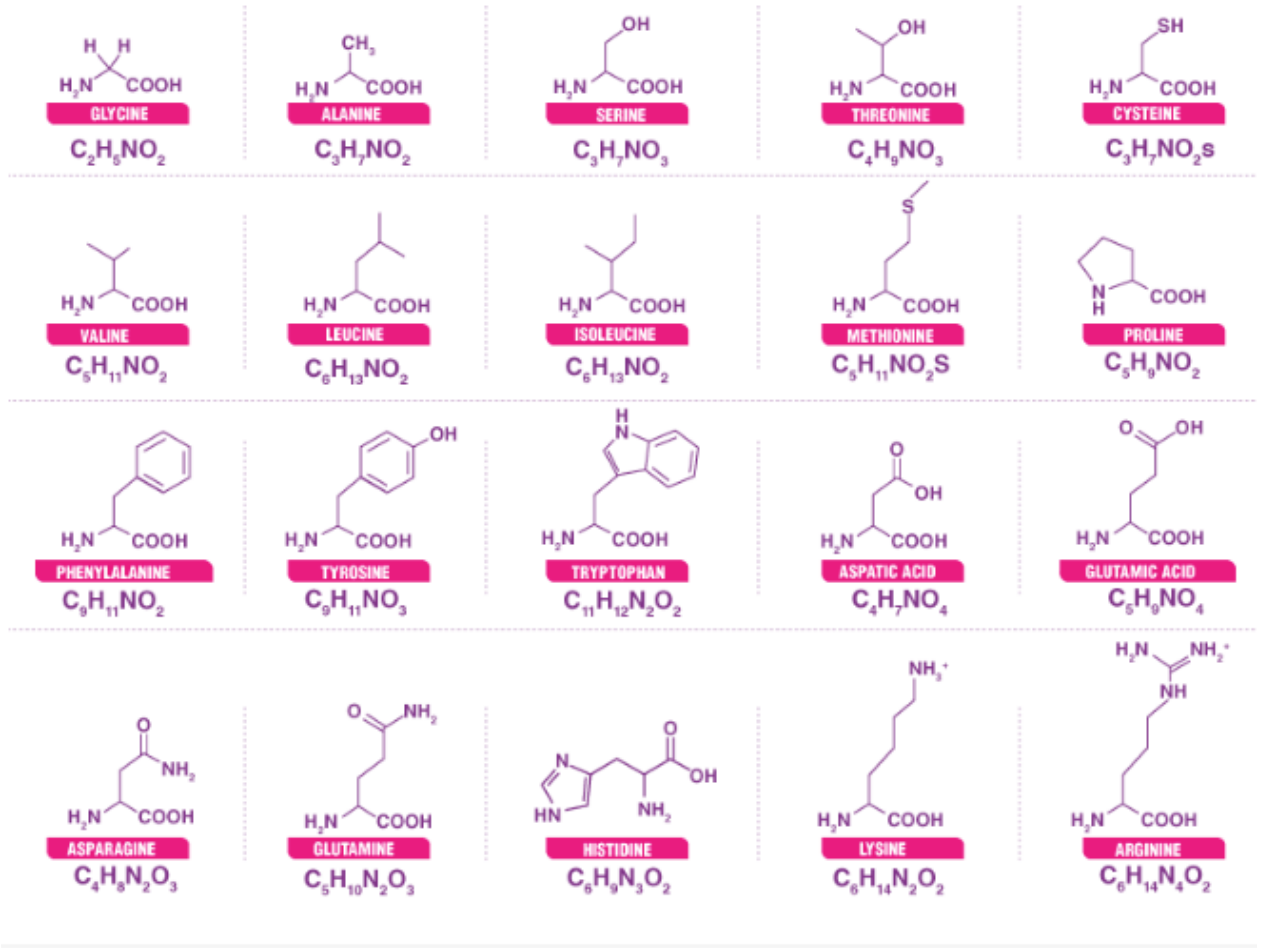
Amino Acid Basics (cont'd.)

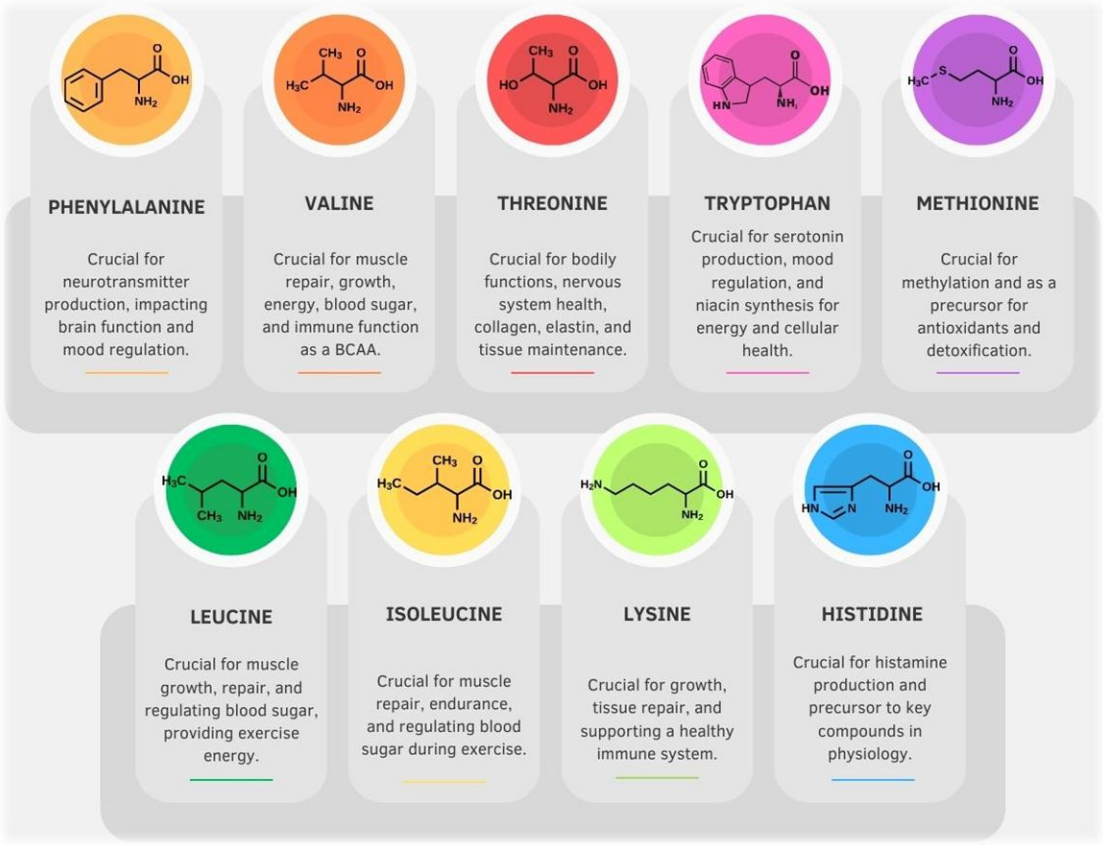
500

Amino acids found in nature, but….



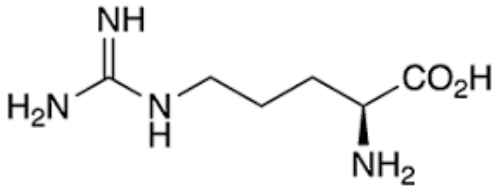
Only 20 are found in the human body

Amino Acid Basics (cont'd.)

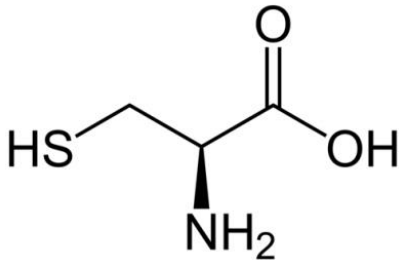


Essential Amino Acids

Two other amino acids become essential when the body is under stress or after trauma:



Arginine



Cysteine

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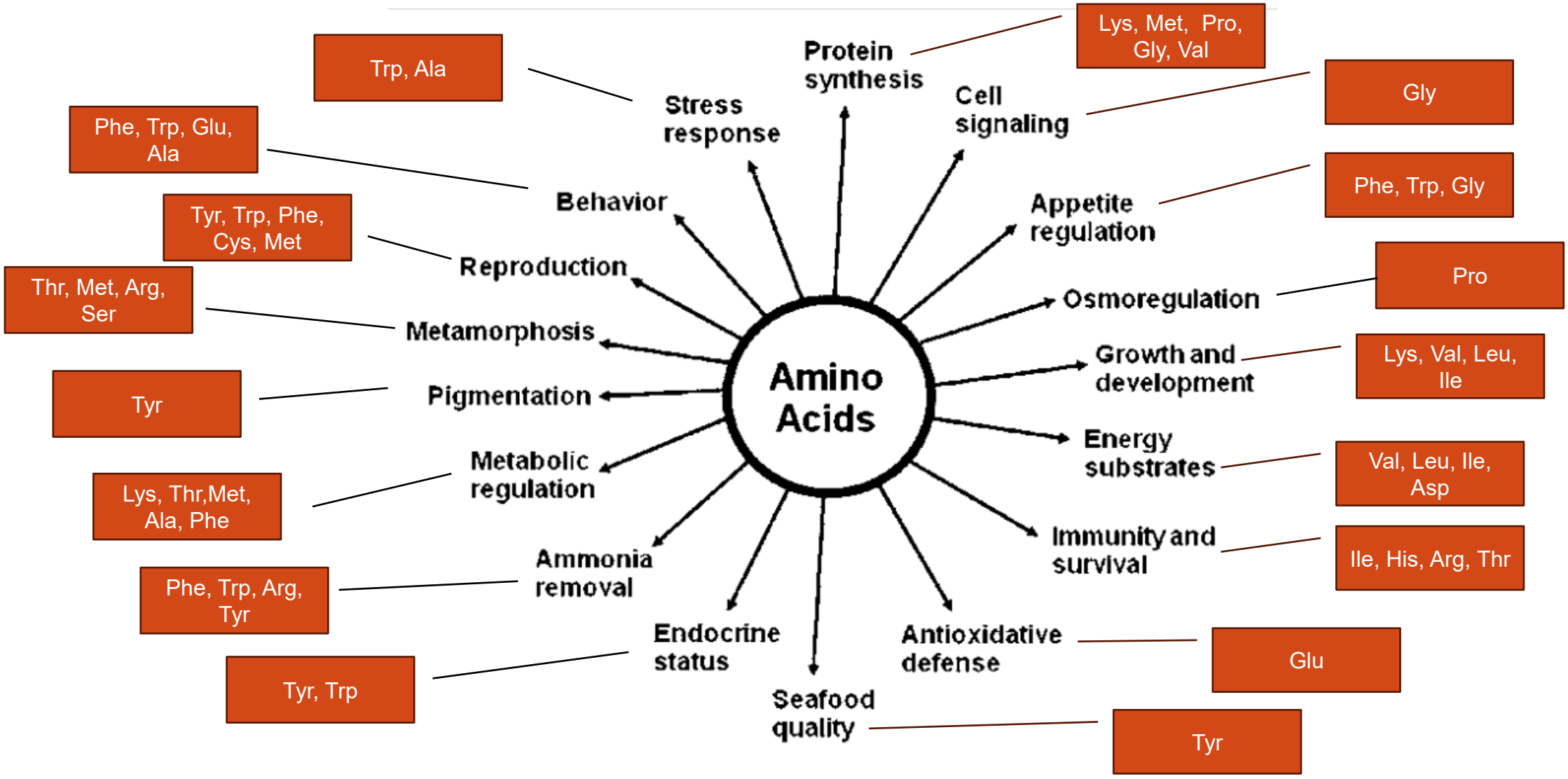
Physiological Effect of Amino Acids



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Fun Facts about Amino Acids' Roles

Amino Acid(s)	Role
Thr	Aids in fat digestion
Gln, Ala	Aids in metabolism of alcohol
Tyr	Responsible for hair, eye, and skin color
Val, Leu, Ile	BCAA - Muscle builders
Arg	Maintains blood flow in veins; removes ammonia from the body
Ala	Improves liver functionality
Pro	Assists with skin moisturization
Ser	Synthesizes precursors for fats

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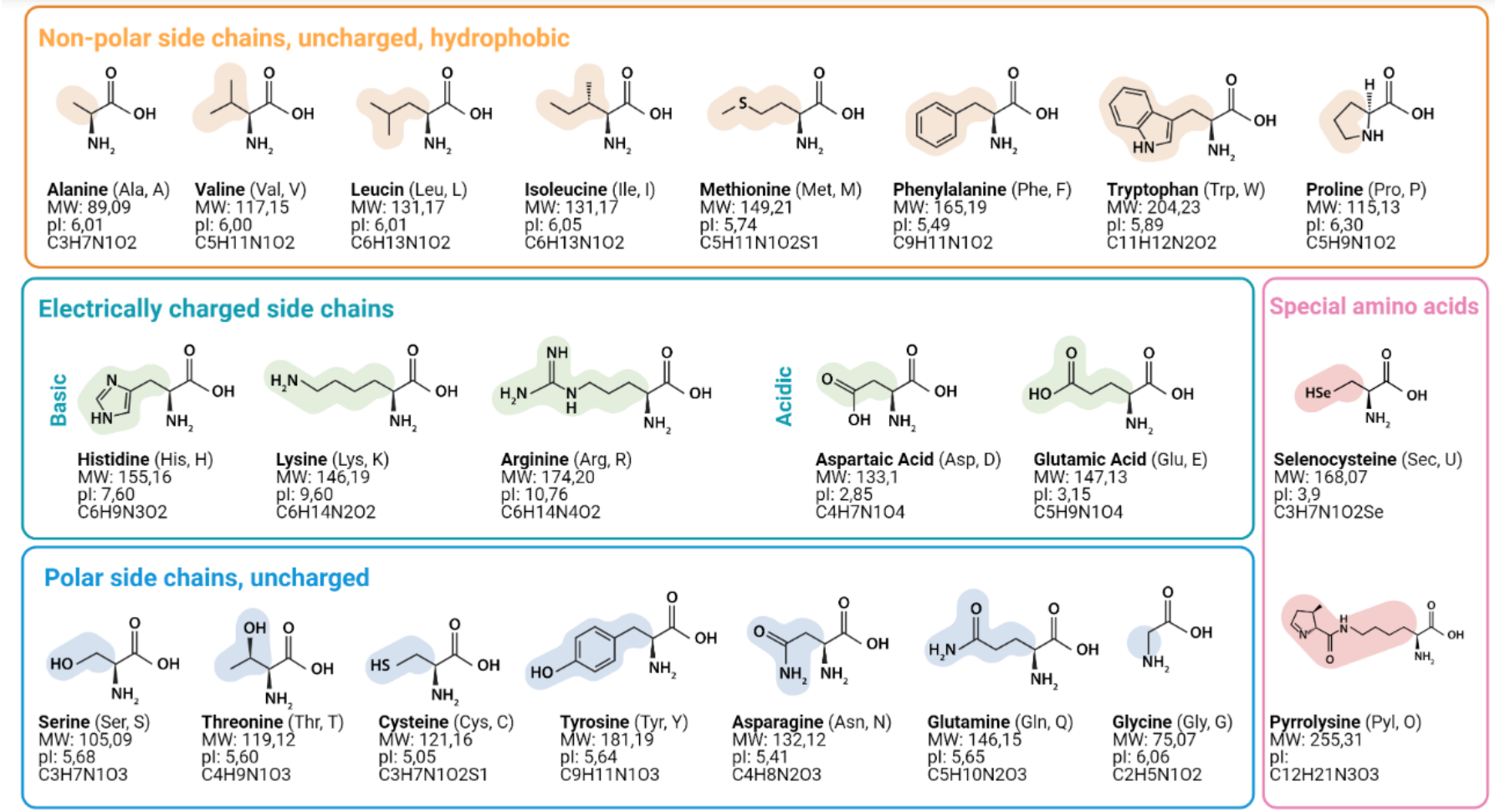
Amino Acids' Properties Influence Physical Behavior of Protein Molecules



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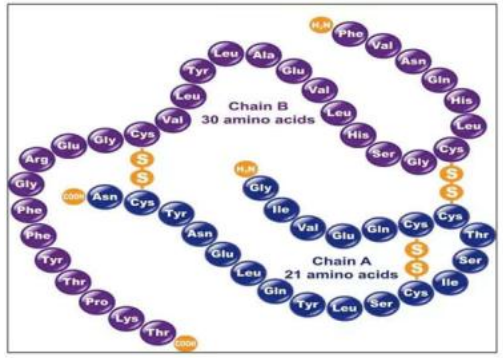
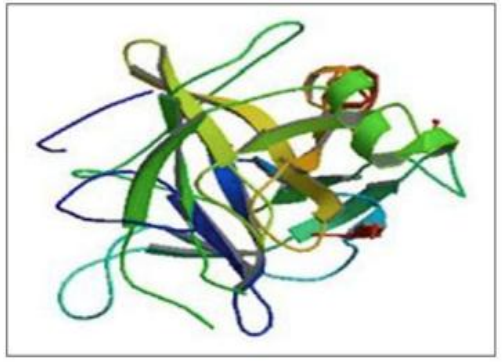
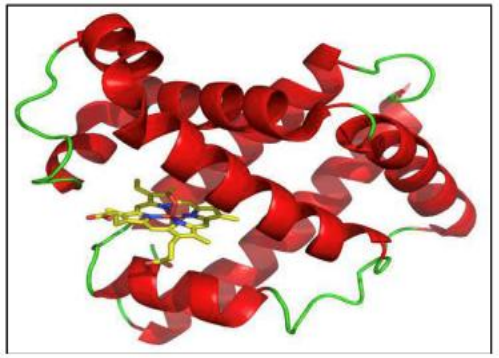
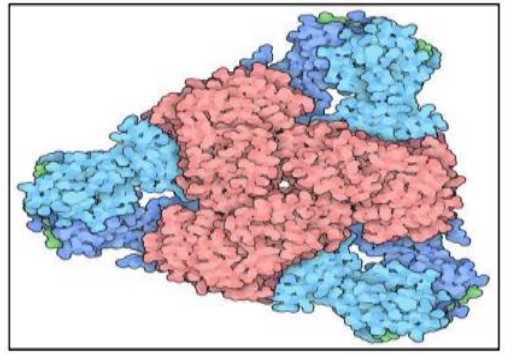
Protein Structure









Primary

Insulin
30 amino acids
3 disulfide bonds

Secondary

Chymotrypsin
245 amino acids
5 disulfide bonds

Tertiary

Myoglobin
153 amino acids
0 disulfide bonds

Quaternary

Aspartate transcarbamylase
310 amino acids
0 disulfide bonds

Ref.: Alhalmi et al. (2020) Intracellular protein biosynthesis: A review. ***Asian J Biochem, Genetics & Molec. Biol.*** 5(2):10-18.

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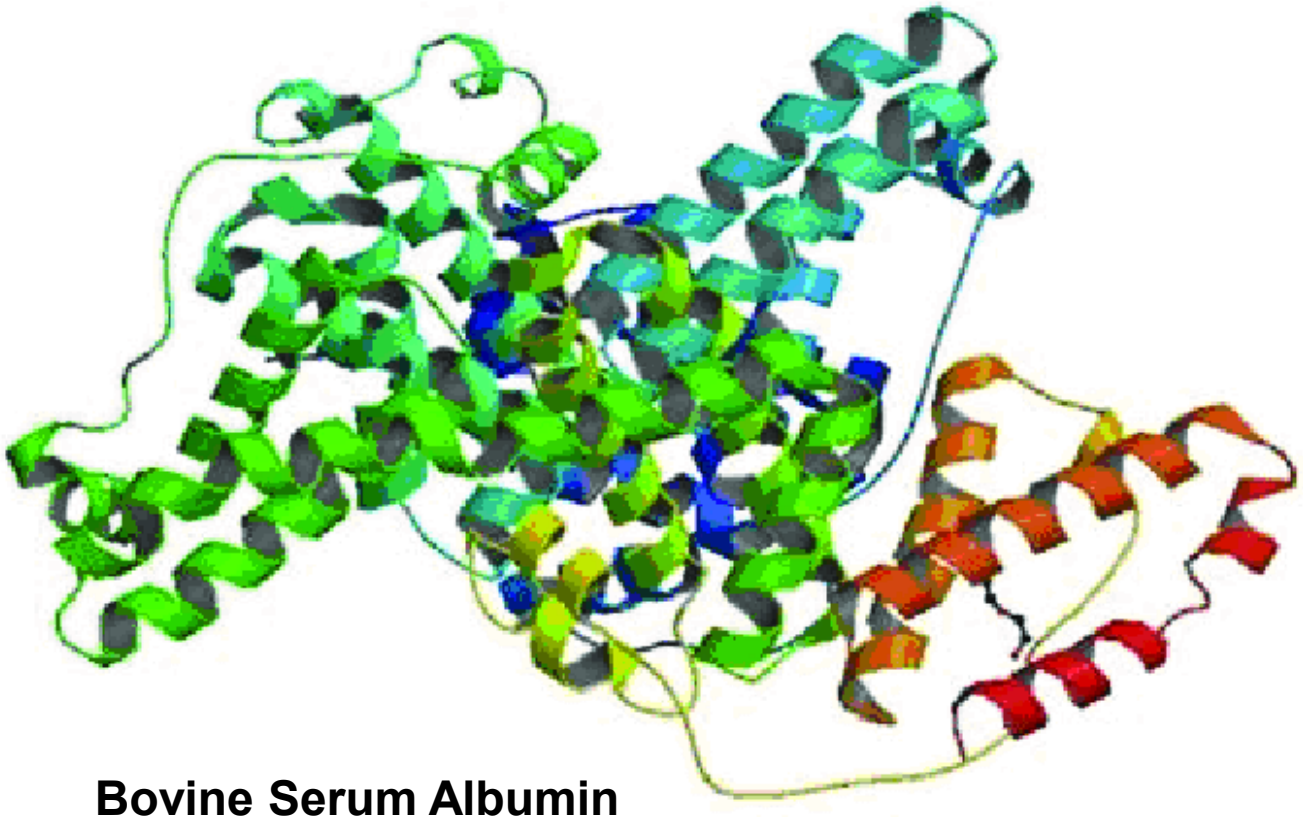
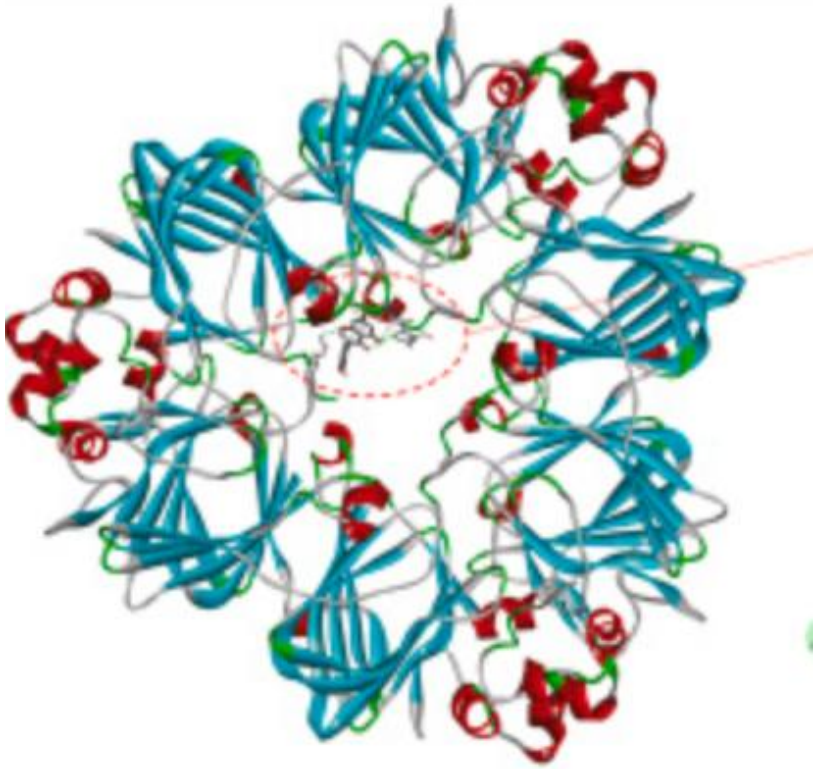
3D Molecular Structure of Animal and Plant Proteins



Bovine Serum Albumin



Soybean glycinin

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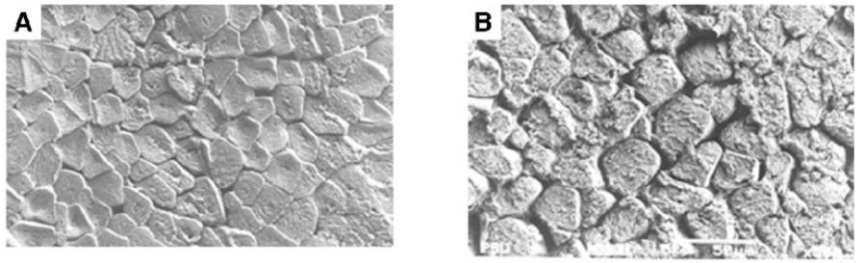
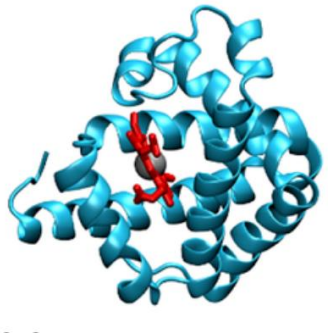
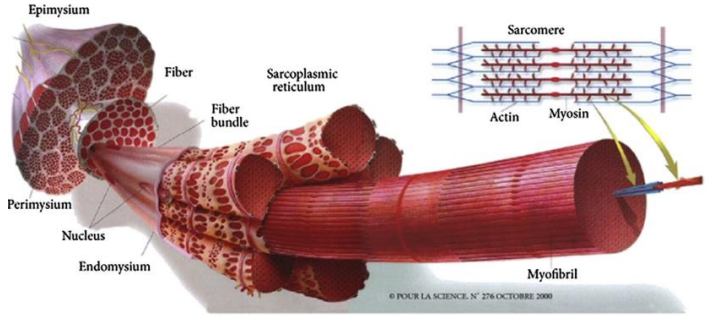
Structure of Animal Protein (Meat)



Ref.: Wattanachant, S. et al. (2005). Microstructure and thermal characteristics of Thai indigenous and broiler chicken muscles. **Poultry Science** 84. DOI: 10.1093/ps/84.2.328



Ref.: Eaton, W.A. (2021). Impact of conformational substrates and energy landscapes on understanding hemoglobin kinetics and function. **J. Biol. Physics** 47. DOI: 10.1007/s10867-021-09588-3.



Ref.: Listrat, A. et al. (2016). How muscle structure and composition influence meat and flesh quality. **Scientific World Journal** 2016. DOI: 10.1155/2016/3182746

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The Consumer is driving change…

Frugality

Cost or supply substitution

More simple/free

Clean and simple labels; free from animal sources, allergens

Performance

Protein addition for nutrition, function or texture

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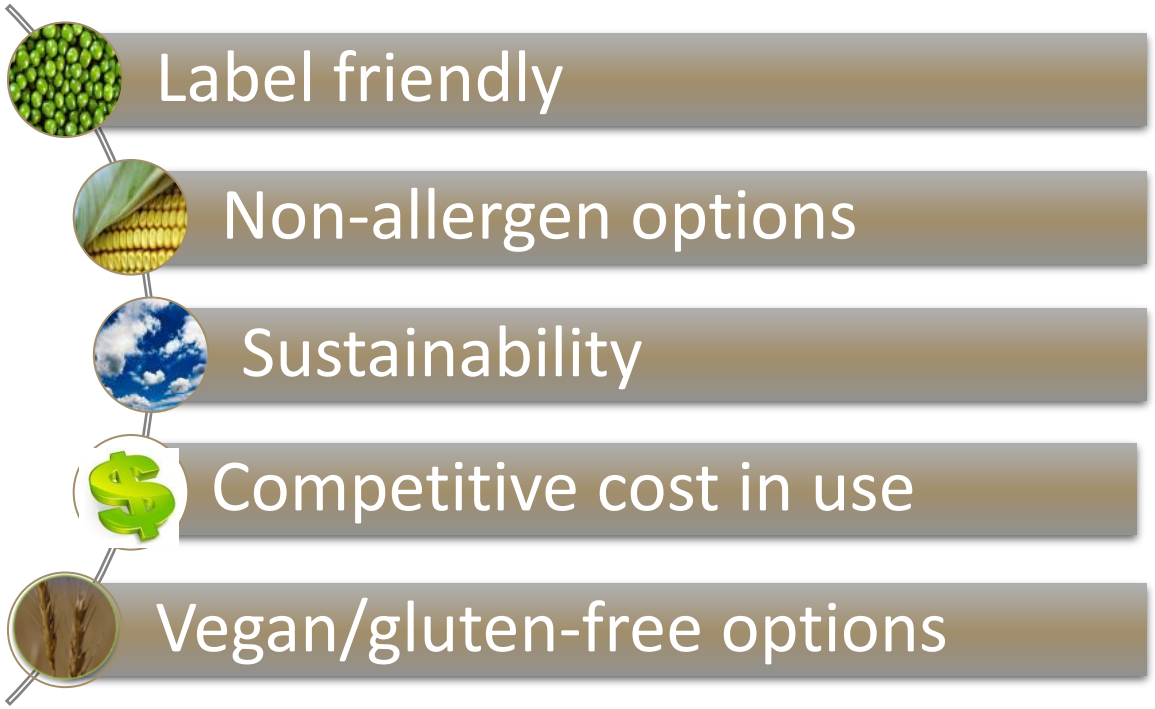
Plant-based proteins have advantages for food and beverage manufacturers



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Protein Selection Criteria

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Key Requirements

Adequate Supply Chain is Imperative

- Grown and processed effectively at commercial scale (kiloton quantities); reasonable cost
- Minimal seasonal or batch-to-batch variation
- Consumers are familiar and comfortable with the source

Process Matters

- Extraction and processing technology is proven at scale and is cost effective.
- The process delivers on the need – high purity, less processed, functionality inducing
- Consumer acceptance
- Microbially and chemically stable for at least 12 months at ambient temperature

Addresses Consumer Wants and Needs

- Initial product offerings have a strong nutritional and functional position
- Supports a path of ingredient and finished product innovation

Imperative for Success

- Texture and Flavor to Sustain Momentum – Good sensory properties maintain the consumer's interest
- Clear regulatory path in major jurisdictions - Inclusion levels align with regulatory acceptance: FDA GRAS and USDA, Health Canada, EU and WHO

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Proximate Composition of Industrially Used Crops for Protein Extraction

Botanical	Moisture (%)	Crude protein (%)	Crude fiber (%)	Crude lipid (%)	Ash (%)	Carbohydrate (%)
Soybean	8.13	39.24	6.84	30.31	4.61	5.08
Wheat	10.76	8.5	7.3	3.03	5.5	64.85
Yellow Pea	6.15	21.7	4.83	2.15	2.88	67.12
White Rice	10.87	5.78	0.89	0.93	0.29	81.24
Mung Bean	9.51	23.2	4.85	31.87	3.67	26.9
Fava Bean	12.3	27.99	13.8	1.57	3.4	40.94
Quinoa	14.7	13.1	3.3	5.7	3.3	59.9

Proximate Composition: Nutritional components of a substance, including its water, protein, lipid, carbohydrate, and ash. Often used to determine the nutritional quality of food.

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Nutritional Value of Proteins

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Essential Amino Acid[1]	RDA[2] (mg/Kg body wgt.)	Soy Protein Isolate (g/100 gm of soy)	Pea Protein Isolate (g/100 gm of pea)
Phenylalanine	33	5.6	4.2
Valine	24	5.4	4.0
Tryptophan	5	1.3	0.7
Threonine	20	4.2	2.8
Isoleucine	19	5.2	3.7
Histidine	14	3.4	1.9
Arginine	No guidance	7.7	6.6
Leucine	42	8.2	6.4
Lysine	38	6.8	5.7
Cysteine	19	2.5	0.8
Tyrosine	33	4.2	3.1

[1]Bolded amino acids are essential for humans for survivability

[2]RDA = Recommended daily allowances

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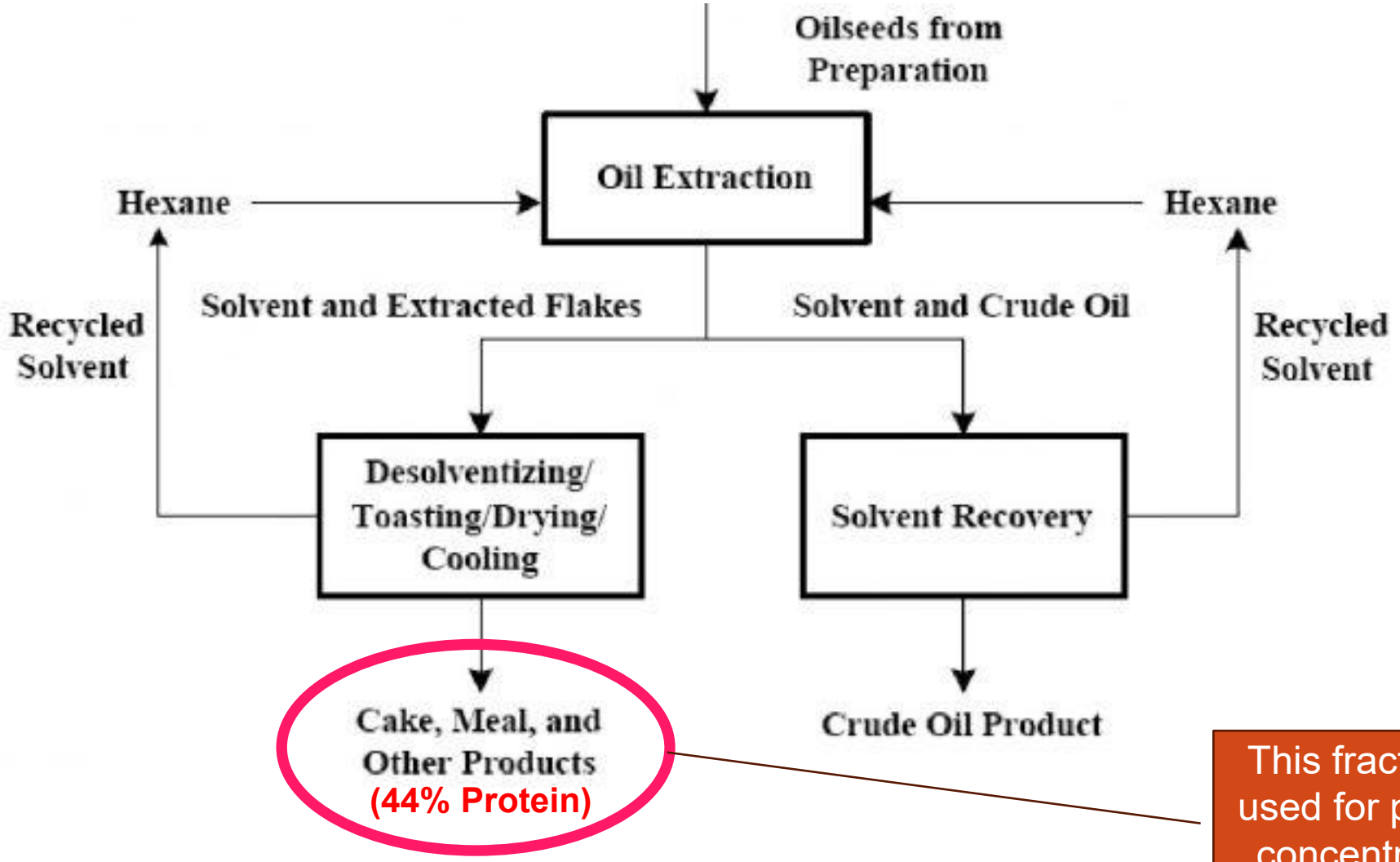
Oil and Oilseed Processing

Harvest/ Pre-process

Oil Seeds



This fraction is used for protein concentration

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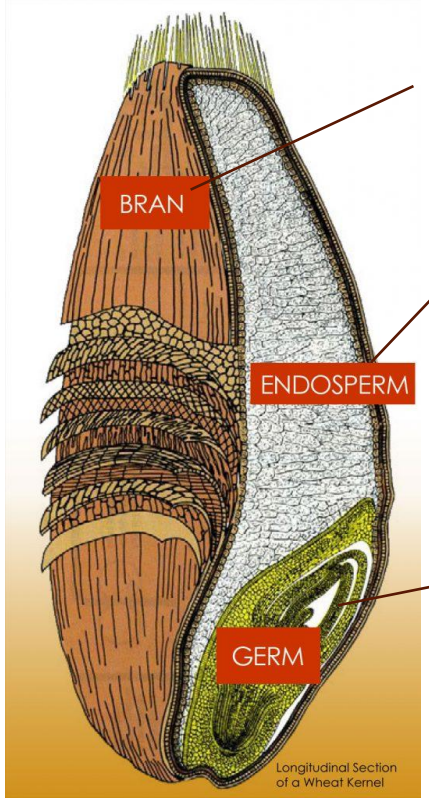
Low Fat Starting Material
Wheat Dry Milling

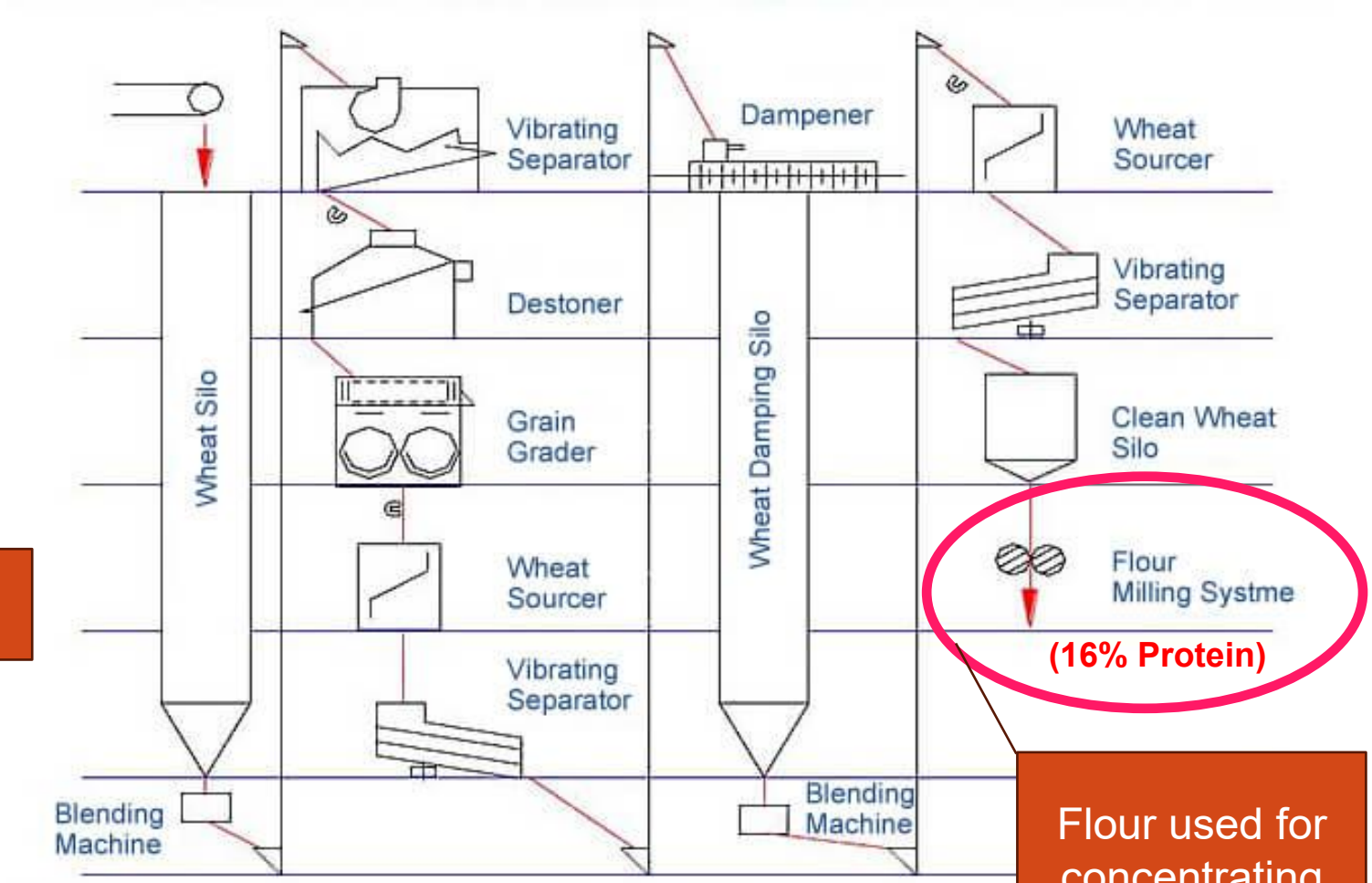
Extraction



16% protein

10.6% protein

31% protein



Flour used for concentrating

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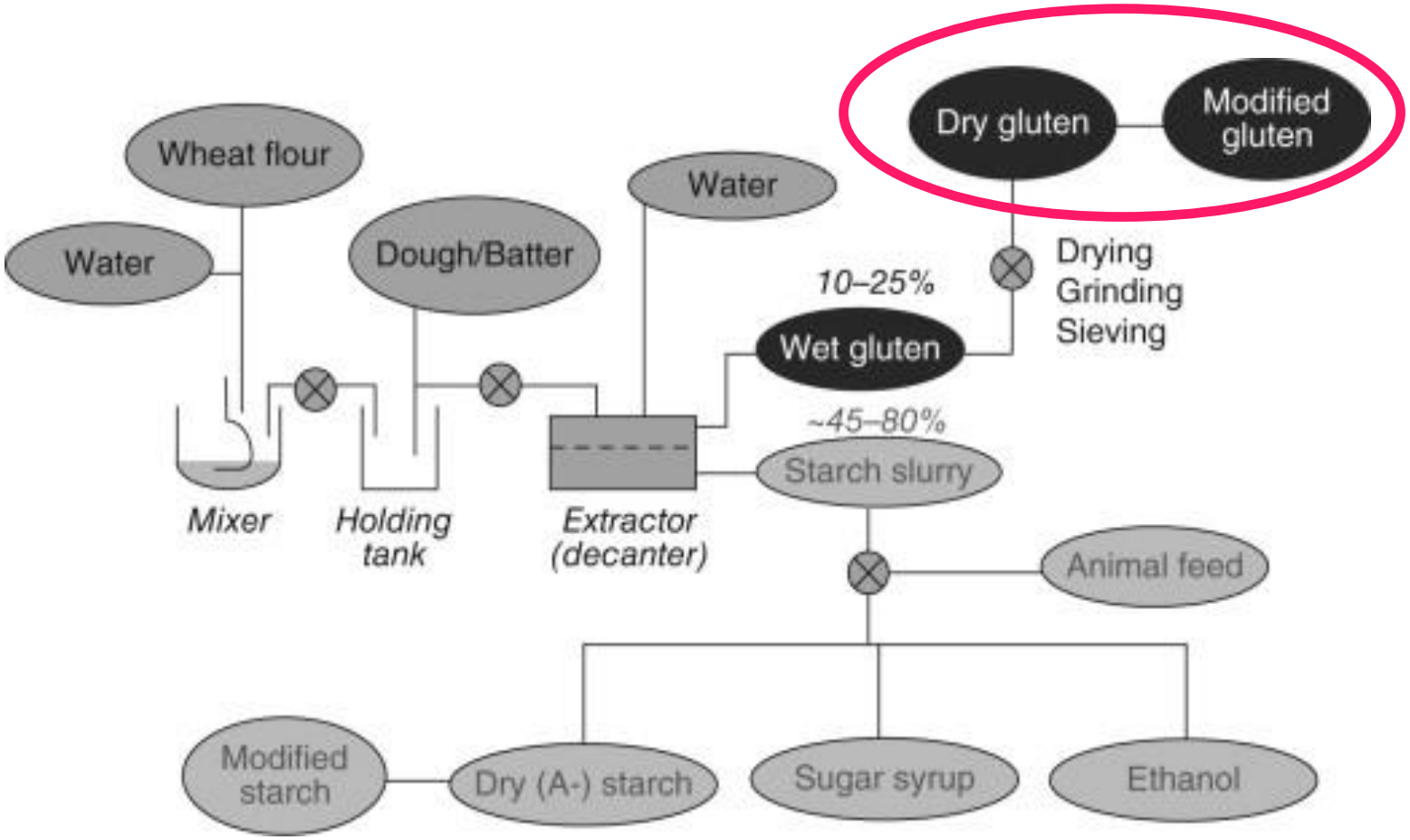
Low Fat Starting Material
Wheat Wet Milling

Concentration



Protein Concentrate
(70% Protein)

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Plant-based Protein Manufacturing Complexity

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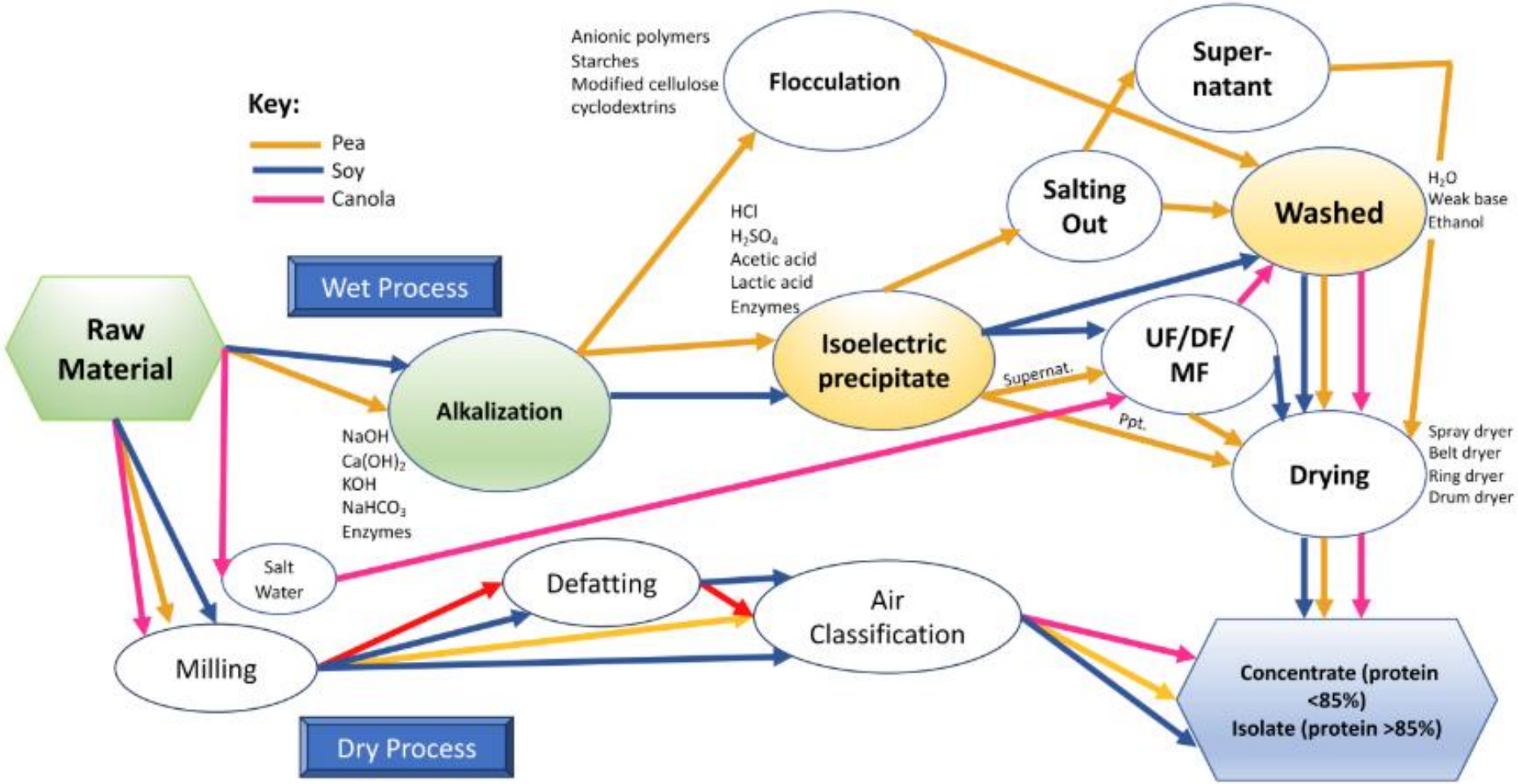

Ref.: Aimutis, W.R. & R. Shirwaiker. (2024) A perspective on the environmental impact of plant-based protein concentrates and isolates. ***Proc. Natl. Acad. Sci.*** DOI:10.1073/pnas2319003121.

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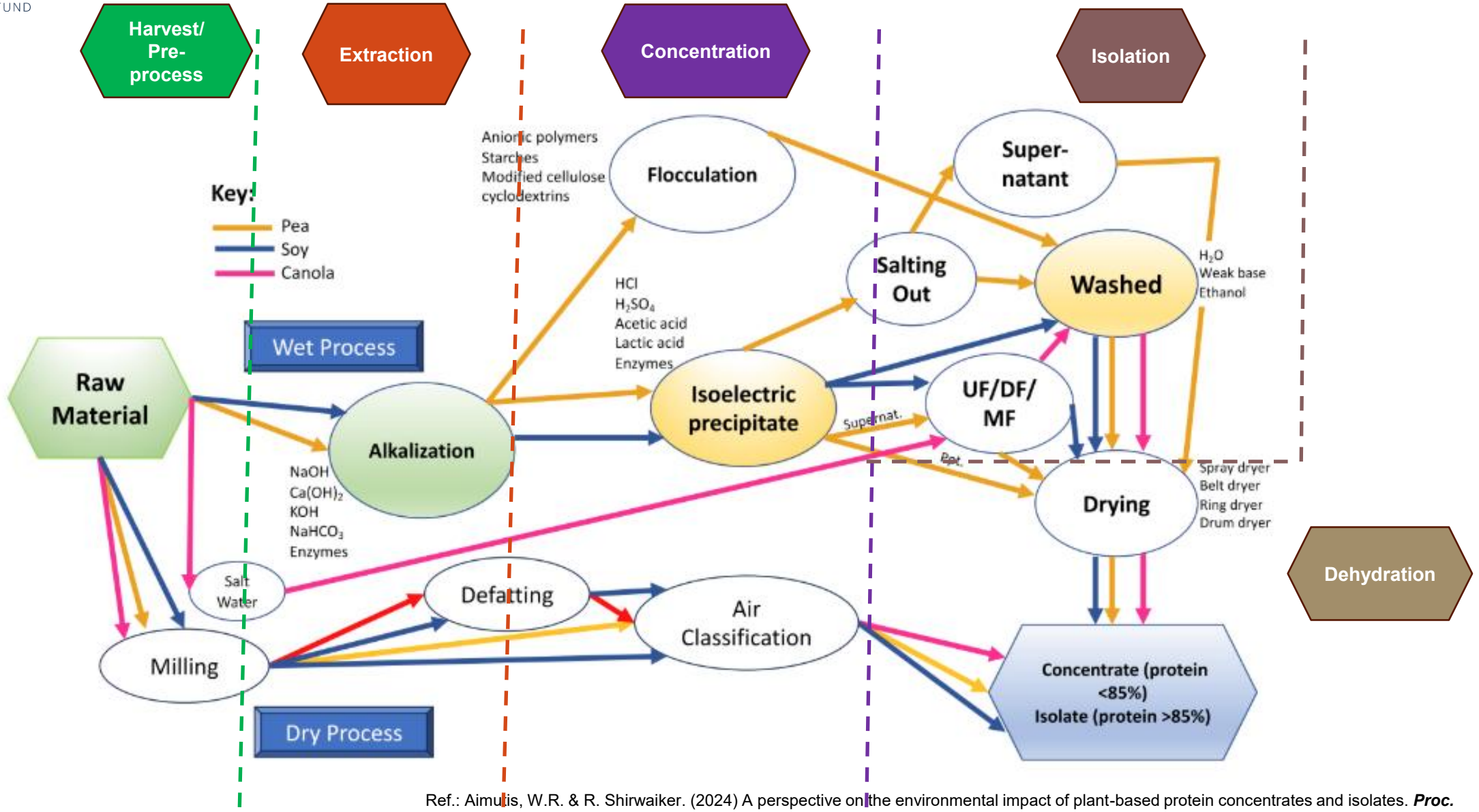

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PBP Extraction Methods

Definition: The goal of protein extraction is to break open plant materials/cells to release their contents that consist of numerous components including starch, fiber, sugars, fats, plant-color components (chlorophyll, polyphenols, and carotenoids), numerous minor components, and of course protein.

HARVEST

Mechanical Extraction Methods

Whole plant
Leaves
Roots
Grains
Seeds

Choice of the proper method(s) is critical to maximize protein extraction with minimal damage to the proteins.

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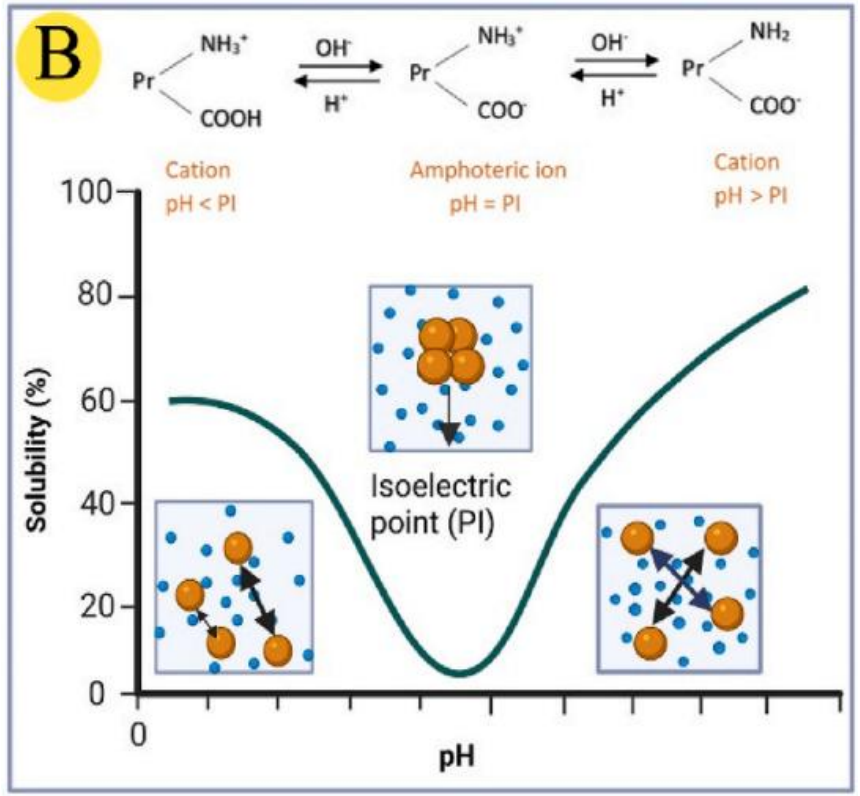
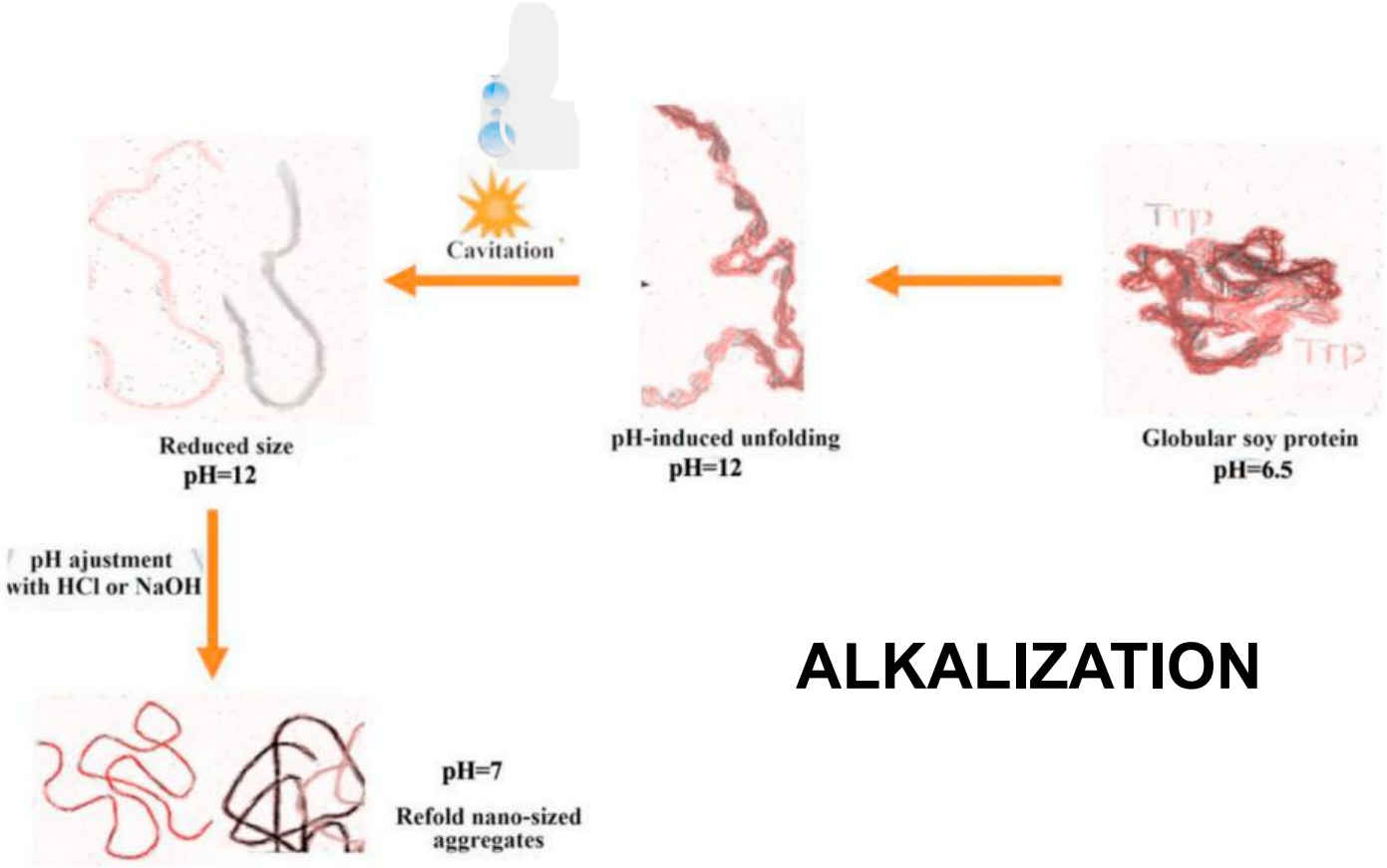
Protein Concentration by pH Adjustment

Concentration





ALKALIZATION

Ref.: Hadidi, M. et al. (2023). Enhanced alkaline extraction techniques for isolating and modifying plant-based proteins. ***Food Hydrocolloids*** 145:109132

Ref.: Momen, S. et al. (2021). Alkali-mediated treatments for extraction and functional modification of proteins: Critical and application review. ***Trends in Food Sci Technol.*** 110:778-797.

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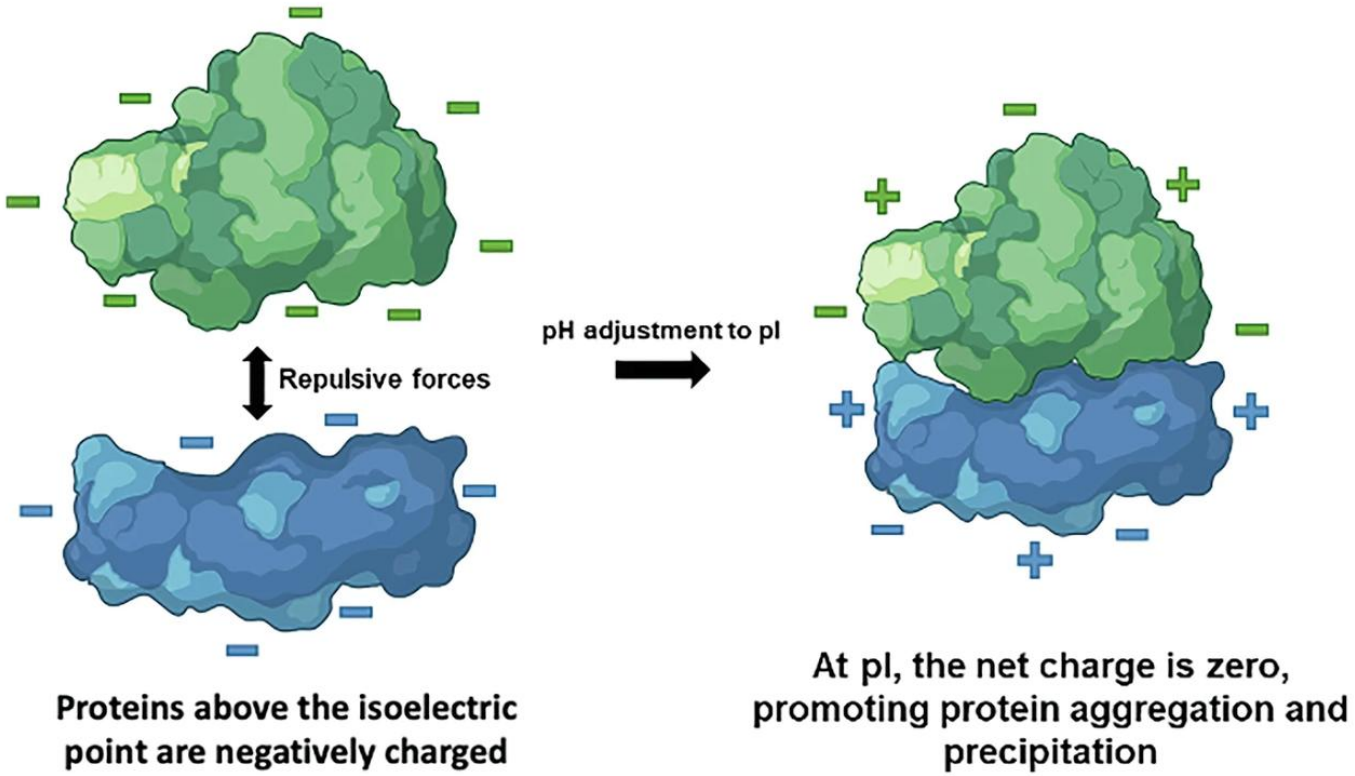
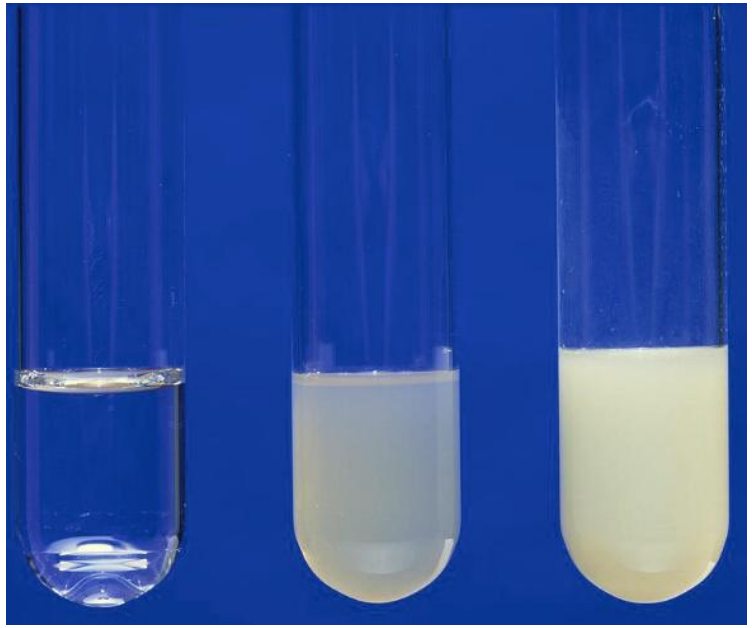
Protein Concentration by pH Adjustment

Concentration



Proteins above the isoelectric point are negatively charged

At pI, the net charge is zero, promoting protein aggregation and precipitation



pH 7 pH 5 pH 4

ALKALINE ENVIRONMENT **ACIDIC ENVIRONMENT**

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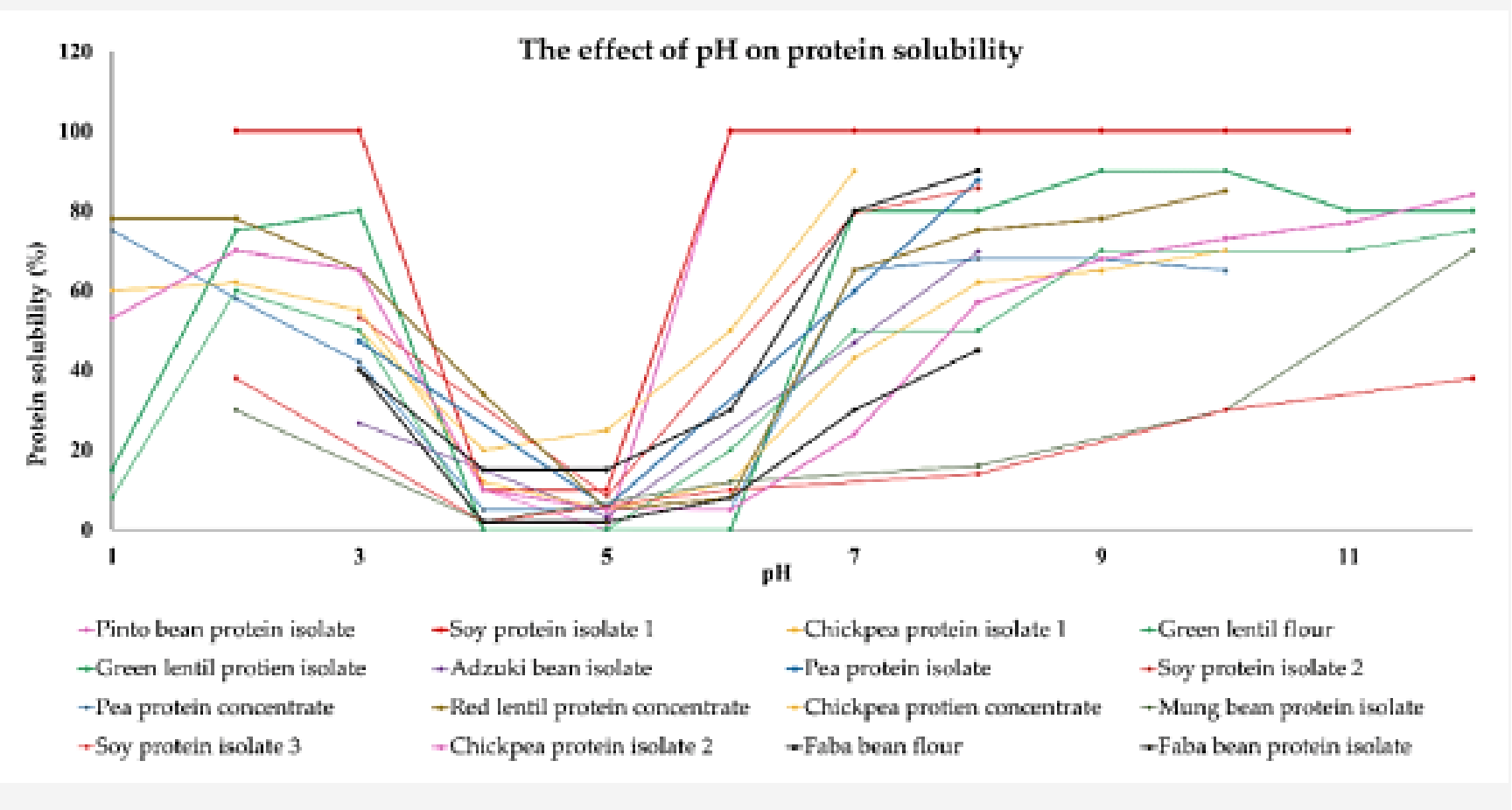
Not All Proteins Behave the Same!

Concentration



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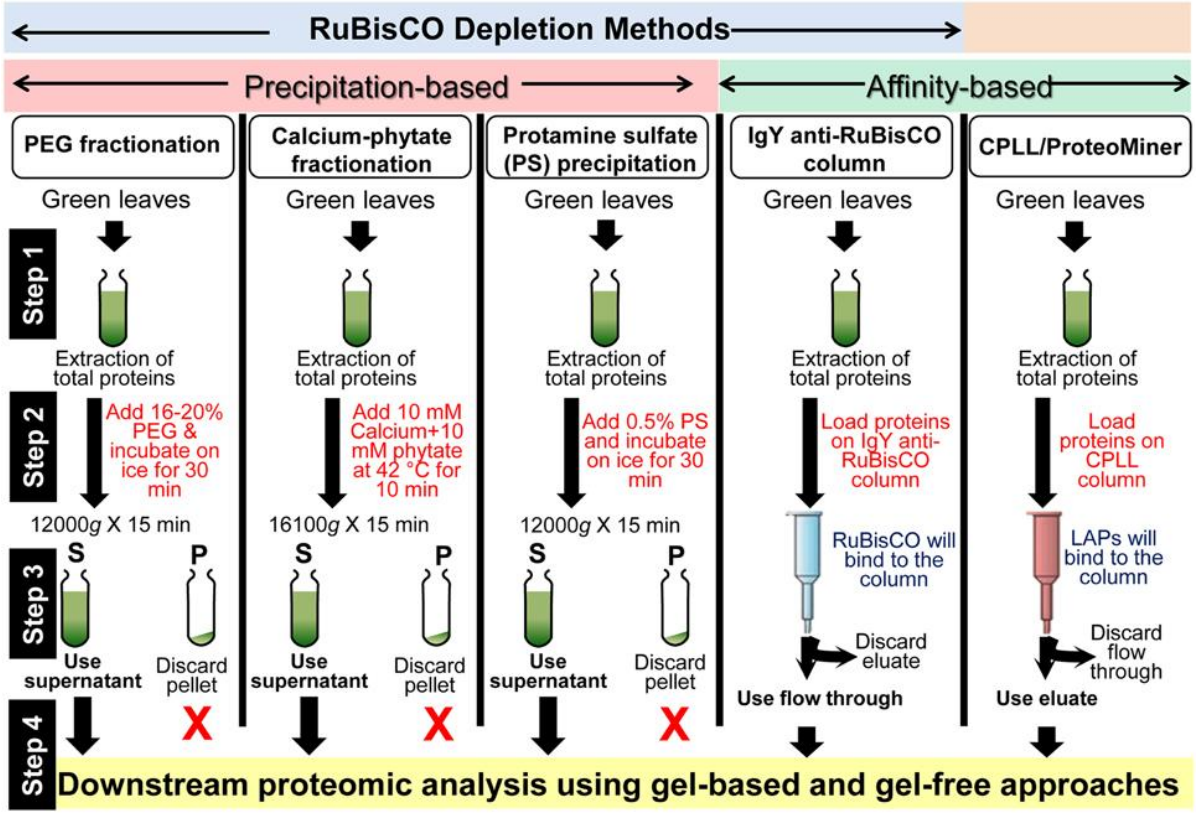
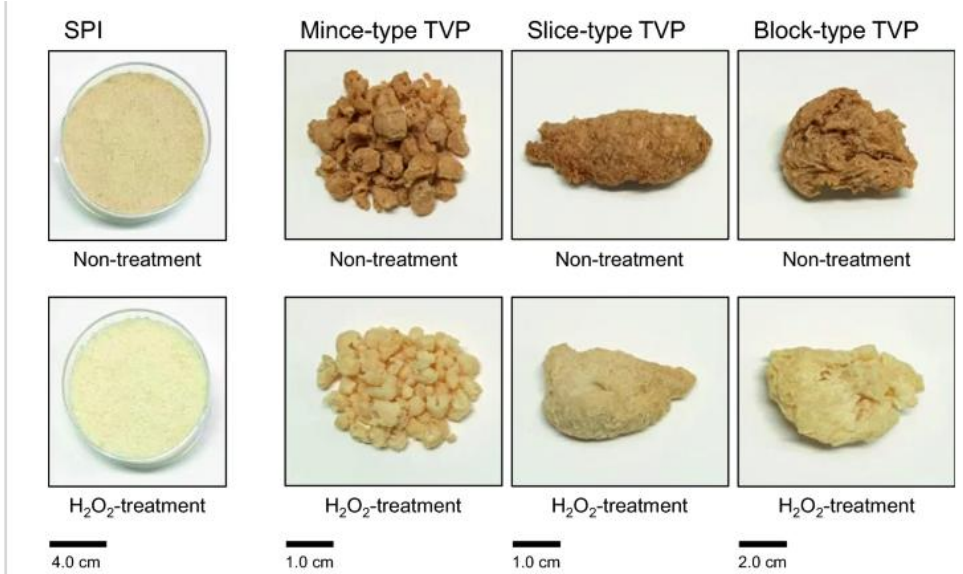
Decolorization of Extracted Proteins

Extraction





Other Methods to Decolorize:

- Hydrogen peroxide / benzoyl peroxide (bleaches)
- Ion exchange resins, e.g. Amberlite™
- Flocculation, e.g. alum (potassium aluminum sulfate)
- Ultraviolet C wavelength irradiation

Ref.: Sakai, K. et al. (2022) Decolorization and detoxication of plant-based proteins using hydrogen peroxide and catalase. ***Scientific Reports*** 12, 22432.

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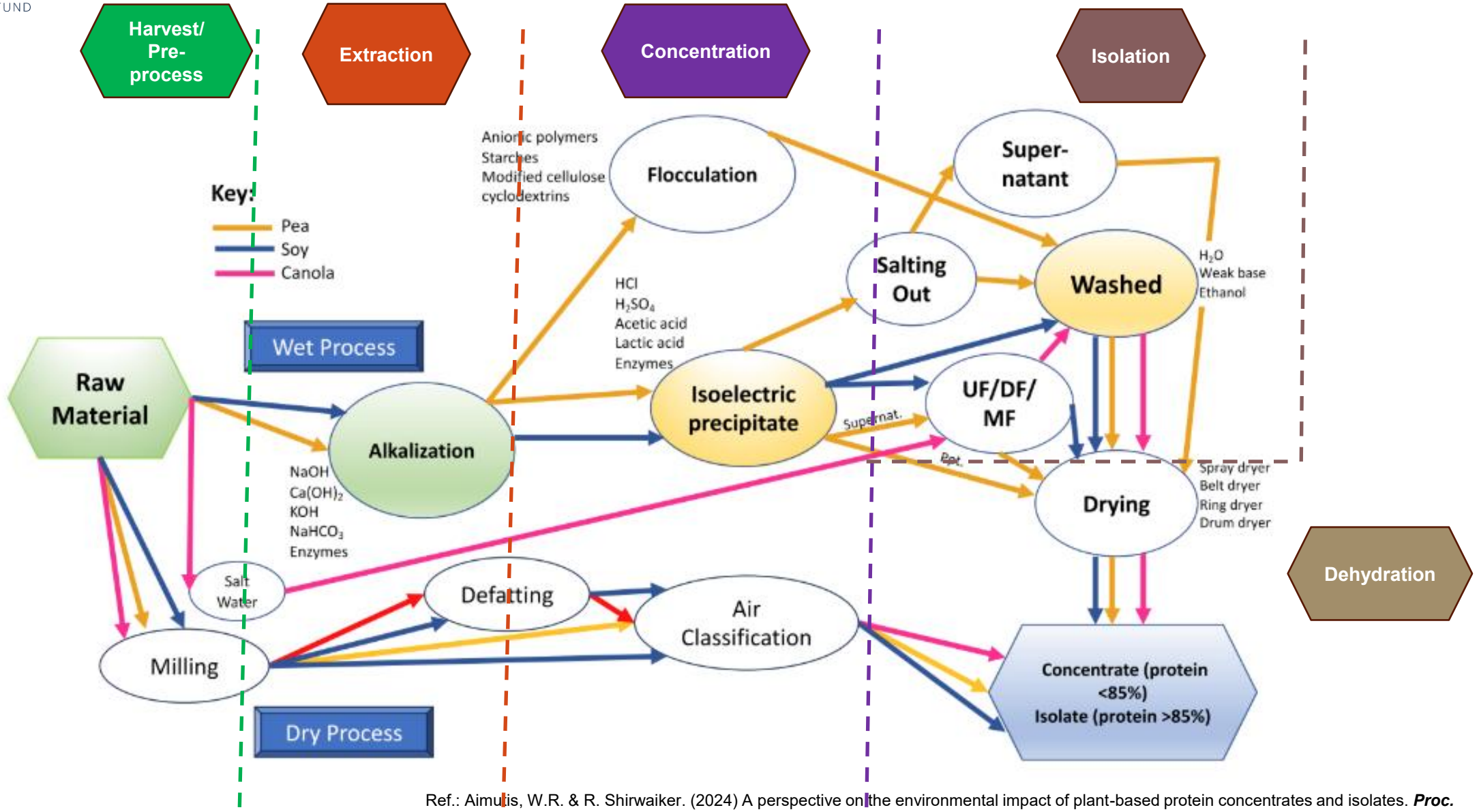
Plant-based Protein Manufacturing Complexity



Ref.: Aimutis, W.R. & R. Shirwaiker. (2024) A perspective on the environmental impact of plant-based protein concentrates and isolates. ***Proc. Natl. Acad. Sci.*** DOI:10.1073/pnas2319003121.

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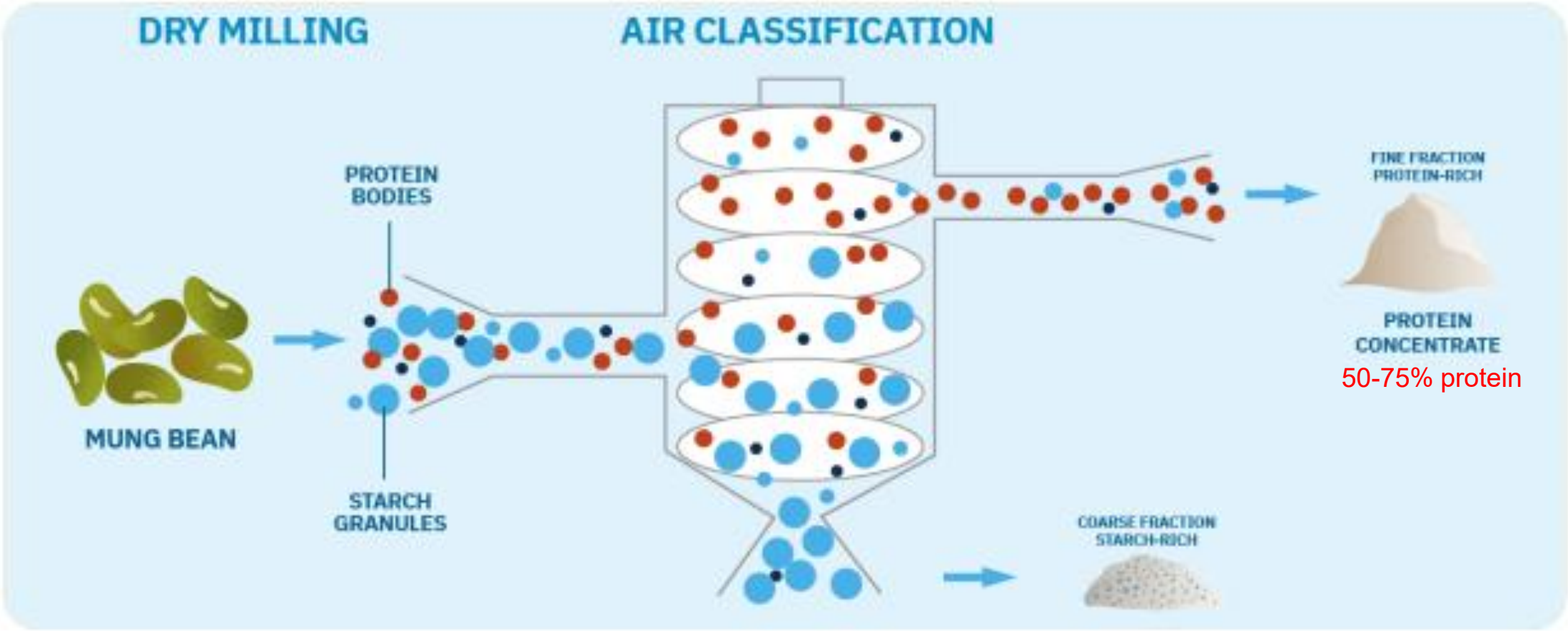
An Alternative: Air Classification

Concentration



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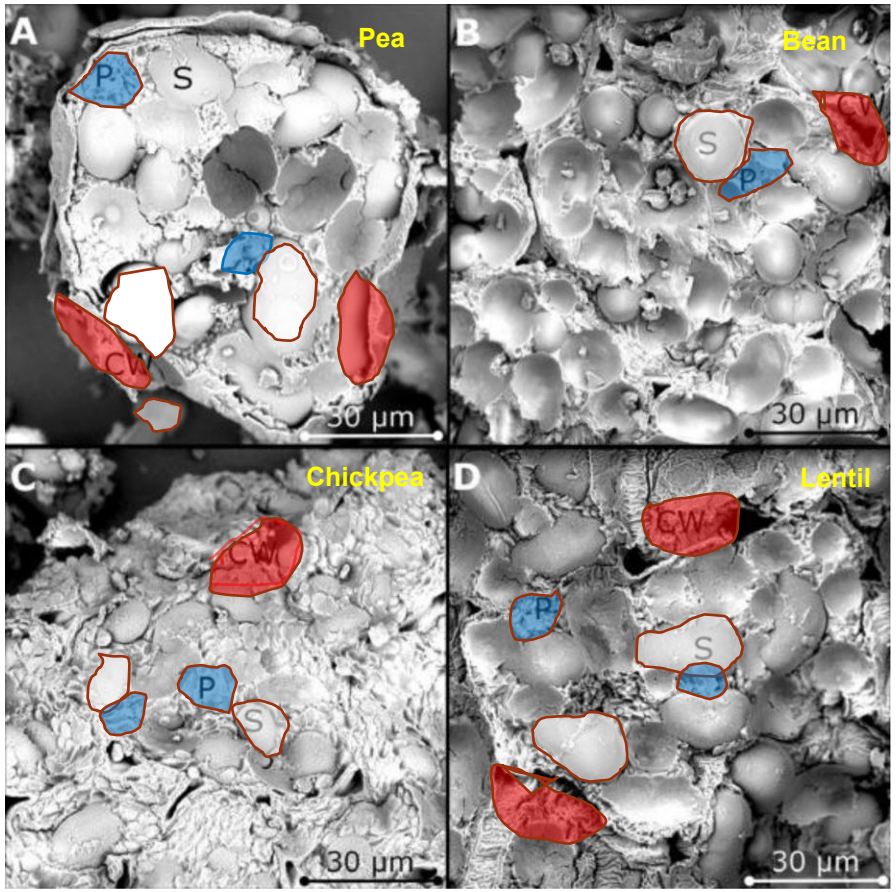
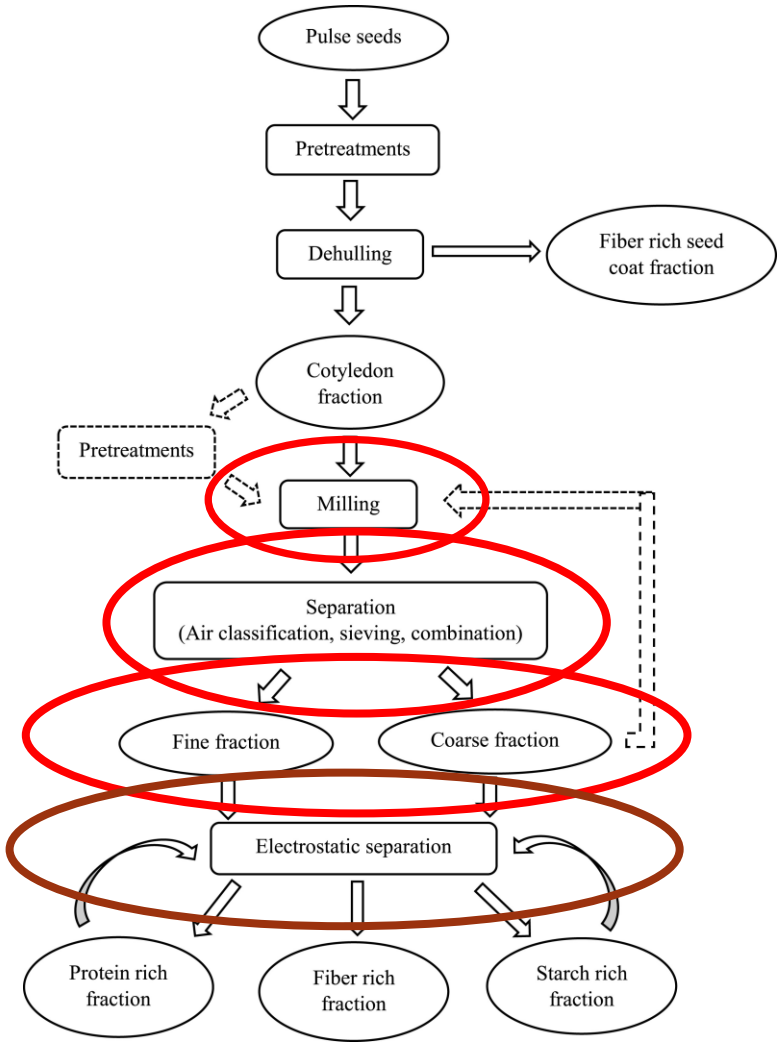
Air Classification

Concentration





Ref.: Fernando, S.(2021) Production of protein-rich pulse ingredients through dry fractionation: A review. *LWT* 141, 110961 doi.org/10.1016/j.lwt.2021.110961

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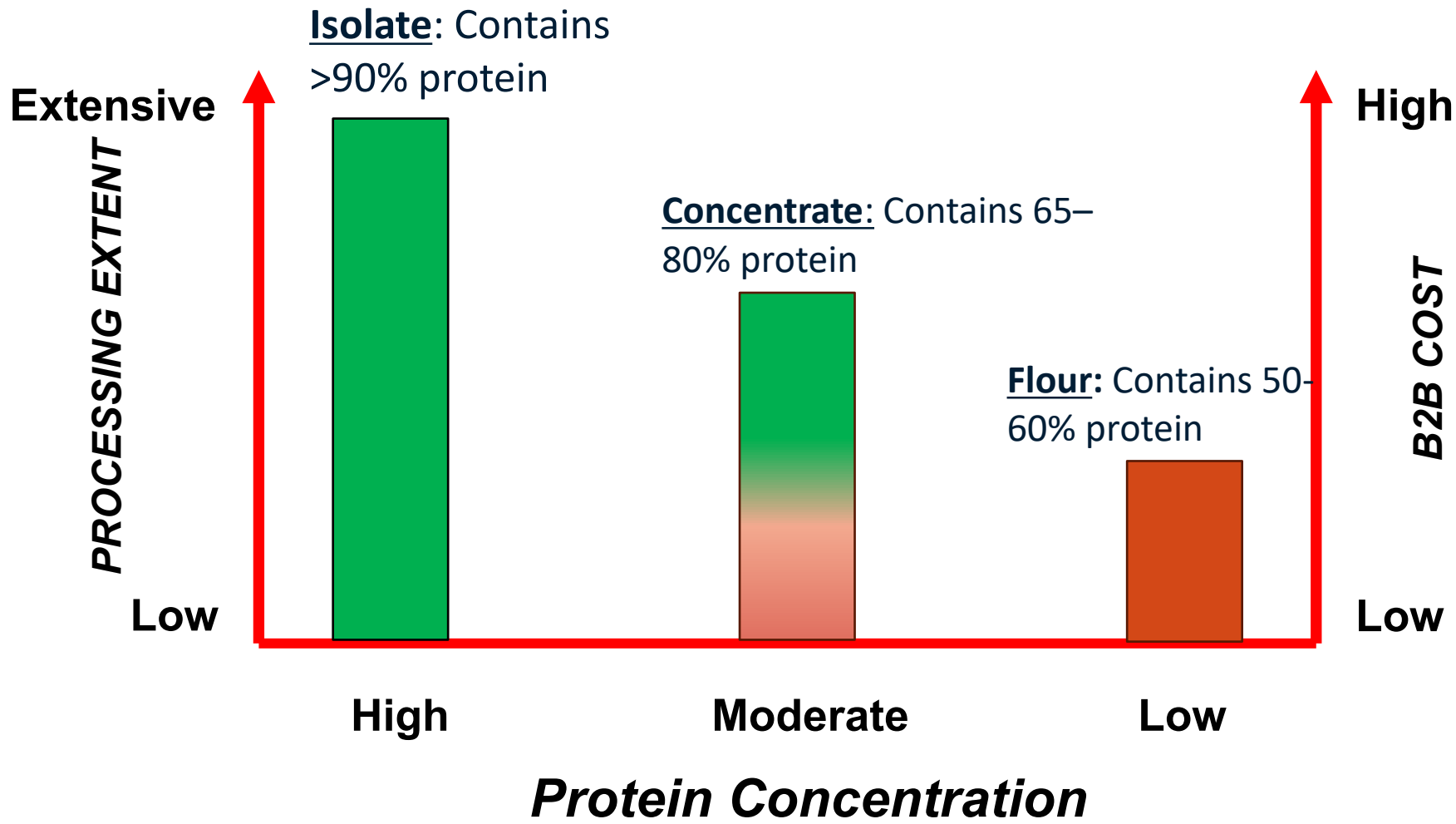
Commercial Protein Formats



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Why do processors add protein to food formulations?

Reasons to use

Functional

Enhanced criteria used by consumer to evaluate food

Appearance: Emulsifying, foaming, water/oil binding, and organoleptic (color/taste/smell)

Flavor: Proteolysis, sweetness, saltiness, accentuation, and solubility

Texture: Water binding, gelation, viscosity, heat stability

Powder characteristics: dispersibility, wettability, flowability

Key Metrics: NSI

Nutritional

Physiological properties linked to protein bioactives

- Improved short-term memory
- Low glycemic response
- Lower PSA counts
- Improved long-term memory
- Improved CV health
- Reduced PMS symptoms
- Better bone health

Key Metrics: PDCAAS; DIAAS

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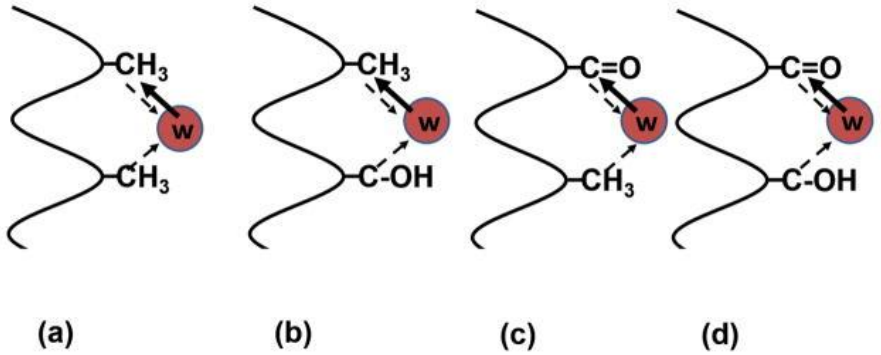
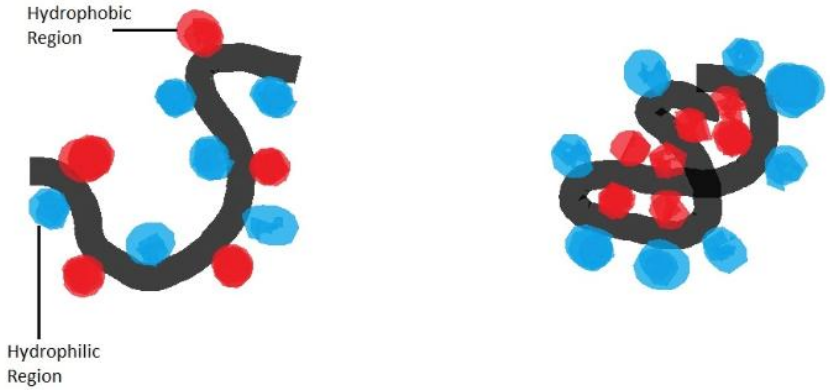
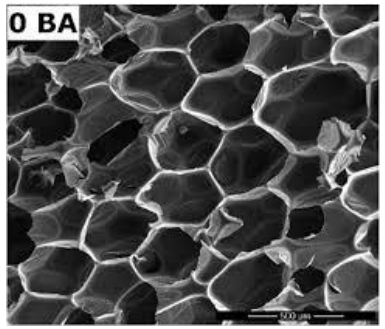
Food Structure Importance of Hydrophobic and Hydrophilic Interactions

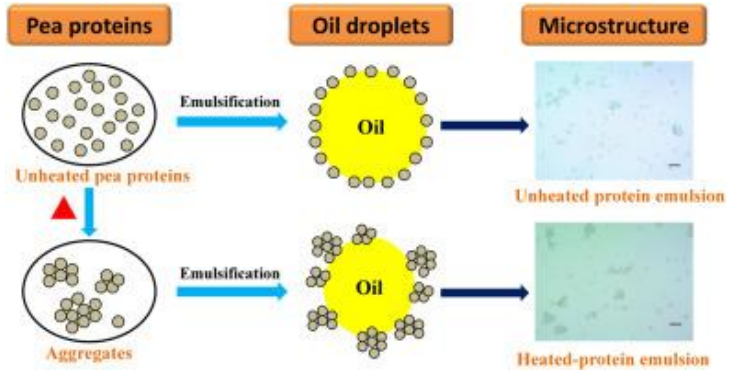


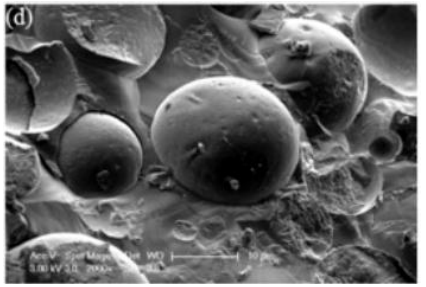
Hydrophilic interactions



Hydrophobic Interactions







Ref.: Durell, S.R. & A. Ben-Naim. (2017). Hydrophobic-hydrophilic force in protein folding. **Biopolymers** 107. DOI: 10.1002/bip.23020.

Ref.: Peng, W. et al. (2016). Effects of heat treatment on the emulsifying properties of pea proteins. **Food Hydrocolloids** 52. DOI:10.1016/j.foodhyd.2015.06.025.

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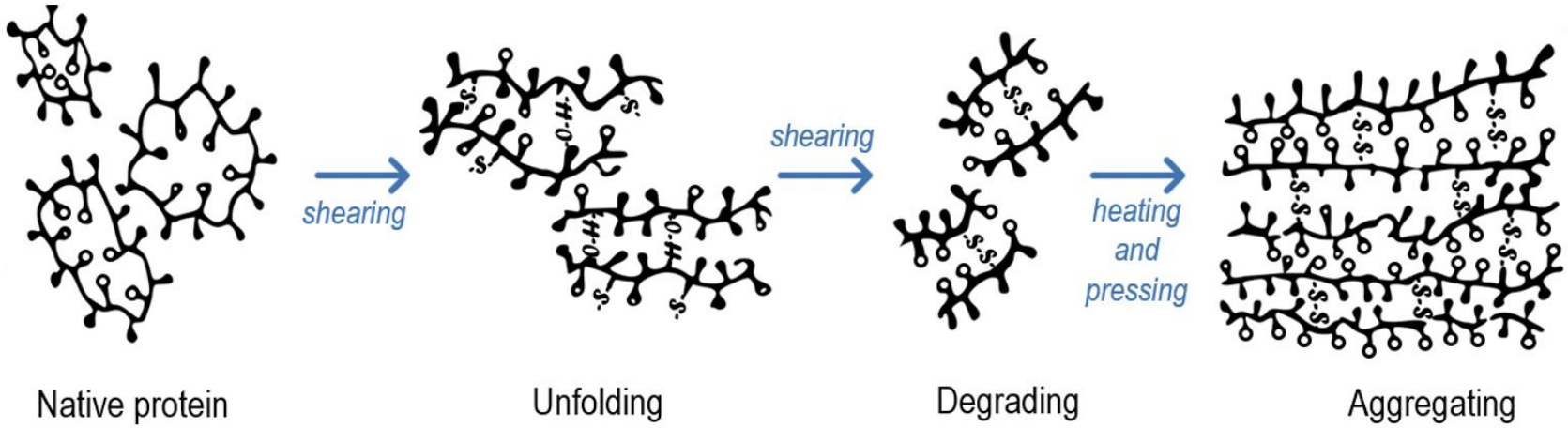
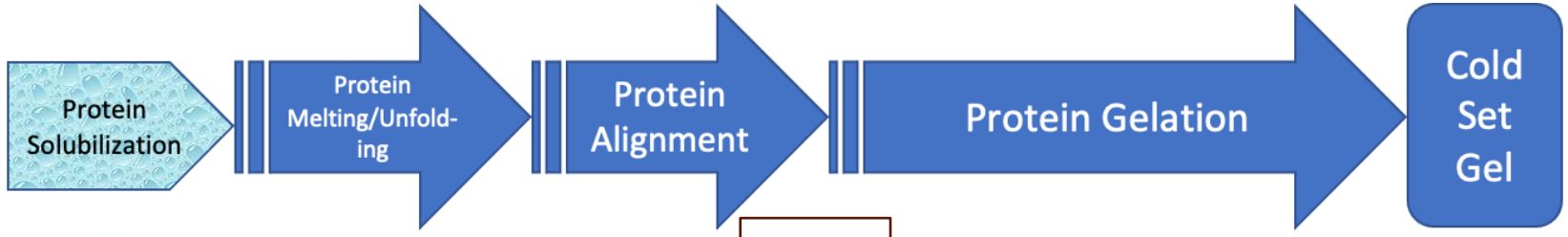
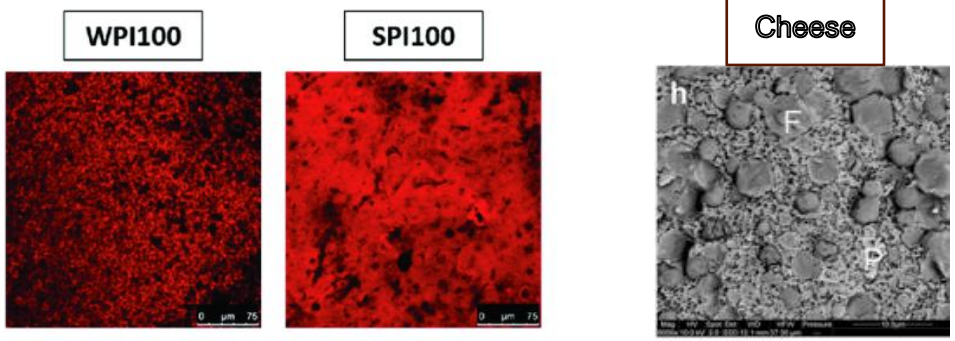
Protein Products Rich in Sulfur Containing and Heat Aggregating Amino Acids







Ref.: Gomez-Mascarque, L.G. (2021). Microstructural analysis of whey/soy protein isolate mixed gels using confocal Ramen microscopy. **Foods** 10. DOI:10.3390/foods1

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Nutritional Value of Proteins

Essential Amino Acid[1]	RDA[2] (mg/Kg body wgt.)	Soy Protein Isolate (%)	Pea Protein Isolate (%)
Phenylalanine	33	5.6	4.2
Valine	24	5.4	4.0
Tryptophan	5	1.3	0.7
Threonine	20	4.2	2.8
Isoleucine	19	5.2	3.7
Histidine	14	3.4	1.9
Arginine	No guidance	7.7	6.6
Leucine	42	8.2	6.4
Lysine	38	6.8	5.7
Cysteine	19	2.5	0.8
Tyrosine	33	4.2	3.1

[1]Bolded amino acids are essential for humans for survivability

[2]RDA = Recommended daily allowances

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Prediction of Protein Digestibility Based on Amino Acid Content

Digestible Indispensable Amino Acid Score (DIAAS) measures protein digestibility in pig ileum (upper small intestine).

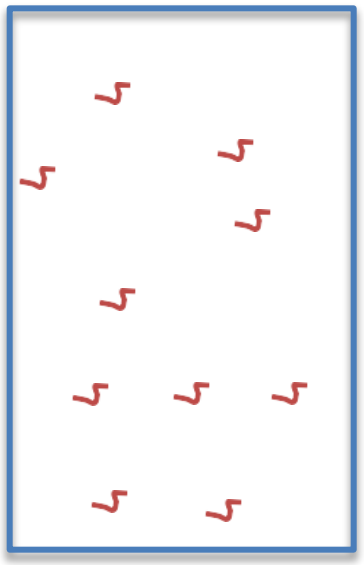
Protein Digesttibility Corrected Amino Acid Score (PDCAAS) uses rat fecal samples to evaluate protein digestibility. Maximum achievable score is 1.0. This method does not account for anti-nuttrients like phtytic acid and trypsin inhibitors which limit protein absorption.

DIAAS is preferred method because it more accurately aligns protein digestibility and amino acid absorption to a human.

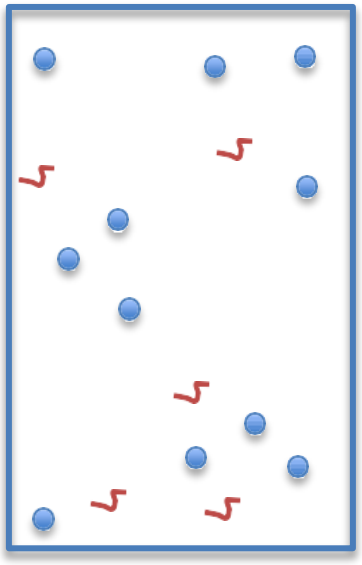
Protein Source	DIAAS (%)	PDCAAS	Limiting Amino Acid
Soy	103	1	NA
Wheat	56	0.4	Lys
Corn	43	0.37	Lys
Rice	56	0.50	Lys
Oat	68	0.57	Lyss
Rapeseed	79	0.83	Lys
Pea	83	0.64	Met + Cys
Canola	85	0.86	Lys
Whey	106	1	NA
Egg	111	1	NA
Casein	137	1	NA
Pork	126	1	NA

Proteins and Phase separation in Beverages

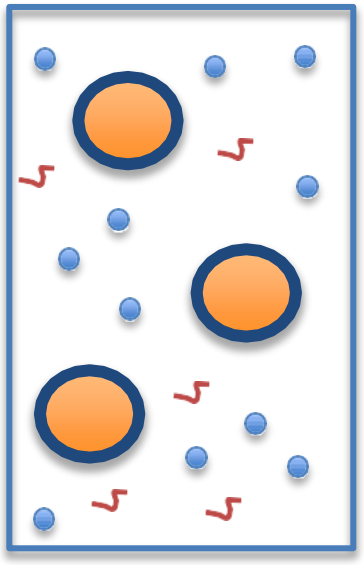
Different length scale levels



Solution
molecular level



Protein dispersion
mesoscopic level



O/W Emulsion
microscopic level

Proteins and Phase separation in Beverages

Protein dispersion
Or
o/w Emulsion

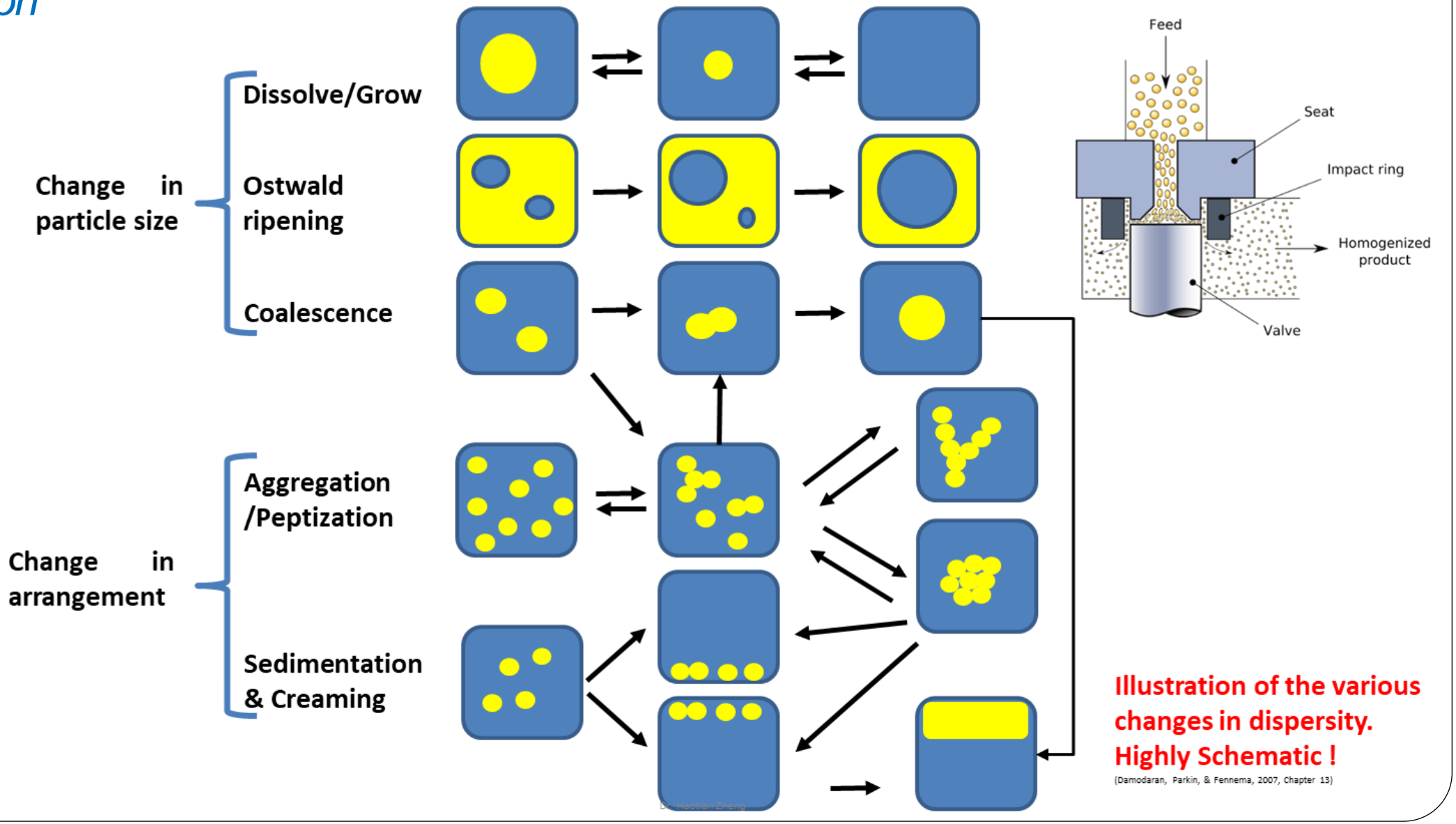


Illustration of the various changes in dispersity. Highly Schematic !

(Damodaran, Parkin, & Fennema, 2007, Chapter 13)

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Thank you