Protein Flavoring Problems: The Whys, Wherefores & Possible Ways Out

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Issues related to flavoring

- Define "flavor"

- Issues with flavoring high protein products
  - Inherent off flavors in the isolates – character and sources
  - Stability of flavors added to high protein foods – loss due to chemical reactions/interactions

- Solutions

What is flavor?

- Summary response of all chemical stimuli which contribute to the perception of flavor

  - Aroma - > 8,500 compounds identified
  - Taste – salty, sweet, bitter, umami, sour
  - Chemesthesis (previously considered trigeminal sense) - heat of spices, cooling of menthol, tingling of spilanthol

Key considerations

- To be sensed - aroma compounds must be free to partition into vapor phase (the breath – aroma) or liquid (saliva in the mouth)

- Much concern about ***interactions*** between food components and flavor since there interactions may bind the flavor so it is not available to be sensed.

Balance of all inputs is required for consumer acceptance

- All senses are interrelated – no sense stands alone.
- All must be balanced to meet human expectations

Aroma – very complex

- Embodies many classes of chemical compounds that vary in reactivity
  - Implications on trying to initially flavor and then maintain flavor during storage
  - **Initial flavor challenge** – perceived flavor is the sum of inherent flavor of the protein used (off notes) and the initial reaction of the flavor with the protein (reactions change the flavor)

Inherent flavor

- What flavor does a plant source bring to a protein isolate and how is it changed by processing and storage?
- Processing and storage - Maillard reactions, lipid oxidation (enzymatic or free radical), enzyme reactions with some component in the plant, etc.
- Look at web comments

Off notes in various protein sources

- Soy protein
  - Beany, green, bitter
- Pea protein
  - Earthy, grassy, nutty, savory; grainy mouthfeel
- Whey protein concentrate (WPC – less protein)
  - Grassy, hay, cheesy, astringent
- Whey protein isolate (WPI)
  - Cardboard, wet dog, cucumber, cooked milk, cabbage, bitter, astringent
- Casein (milk protein)
  - Stale milk, gluey, cheesy, musty, sour
- Protein hydrolysates
  - Astringency, bitterness

- McGorrin, R. 2014 Protein Trends & Technologies Seminar , April 8-9, 2014 • Arlington Heights, IL, USA

“Beany” Flavor in Soy Beans

- Legumes, especially soybeans
  - “beany, grassy, bitter, nutty, green” off notes

- Oxidation - cis-cis double bonded methylene-interrupted fatty acids (oxidation of phospholipids remaining in protein after extraction)

  - Enzymes either initiate or accelerate lipid oxidation depending on enzyme type

- Volatile off flavored compounds formed are:
  - 2-pentyl furan → beany
  - 3-cis-hexenal → green, beany
  - 3-trans-hexenal → oily, grassy
  - Ethyl vinyl ketone → green, beany

Other Soy Off-Flavors

- Bitterness, two sources:

1. Phosphatidyl choline gets oxidized (non enzymatic) to give bitter compounds

2. glucosides of daidzein and genistein (no taste)

↓ Beta glucosidase

daidzein and genistein (bitter)

- Phenolic odors
  - Precursors are ferulic, coumaric, syringic and vanillic acids (normal plant biosynthesis) form 4-vinyl phenol (painty) and 4-vinyl guaiacol (phenolic, bitter)
- Minimize off-flavor
  - Enzyme catalyzed reaction:
    - Processing
    - Soak and denature enzymes
    - Solvent extraction and steam stripping
  - Free radical oxidation:
    - Normal processes to limit oxidation
    - Treatment with cyclodextrin (patent, U Wisc)

“Earthy” notes

- Most commonly associated with geosmin or ….

- Sensory Threshold (water) 3.7-42.2 ng/L
  - ppt threshold

(produced by the gram positive bacteria *Streptomyces*, a genus of Actinobacteria in the order Actinomycetales, and released when these organisms die.)

“Earthy” Note

- 2-methylisoborneol

- Sensory Threshold (water) 10 *n*g/kg
  10 pptr

(Produced by Aspergillus niger, Penicillium aurantiogriseum, and also Penicillium expansum)

Solutions?

- 1. Manufacturer - Changes in processing.
  - Identify the compounds responsible for objectionable odor/taste
  - Determine pathway for formation
  - Develop processes to rid product of these compounds

2. Formulate flavor to use objectionable character

- Can the inherent flavor be a part of the flavor chosen?
  - Bitterness is expected in coffee or chocolate flavor
  - Green grassy can be a part of a fresh fruit flavor
  - Butyric acid (cheesy) or can be part of a product that involves cheese

3. Masking agents

- Great deal of work on masking bitter notes – sugar is a masking agent for bitter – competes for bitter binding sites.
- Progress on bitterness – some … but a very different task to mask off odors (I have not seen success)
- Old saying on the farm – something about making a silk purse out of a sow's ear.

Inherent flavor - summary

- Need to determine what chemicals are inherent to the protein isolates and plan a strategy to remove them.
- Processing challenge

Obtaining/maintaining a desirable flavor in a high protein product

- Issue – flavor “interactions” with proteins

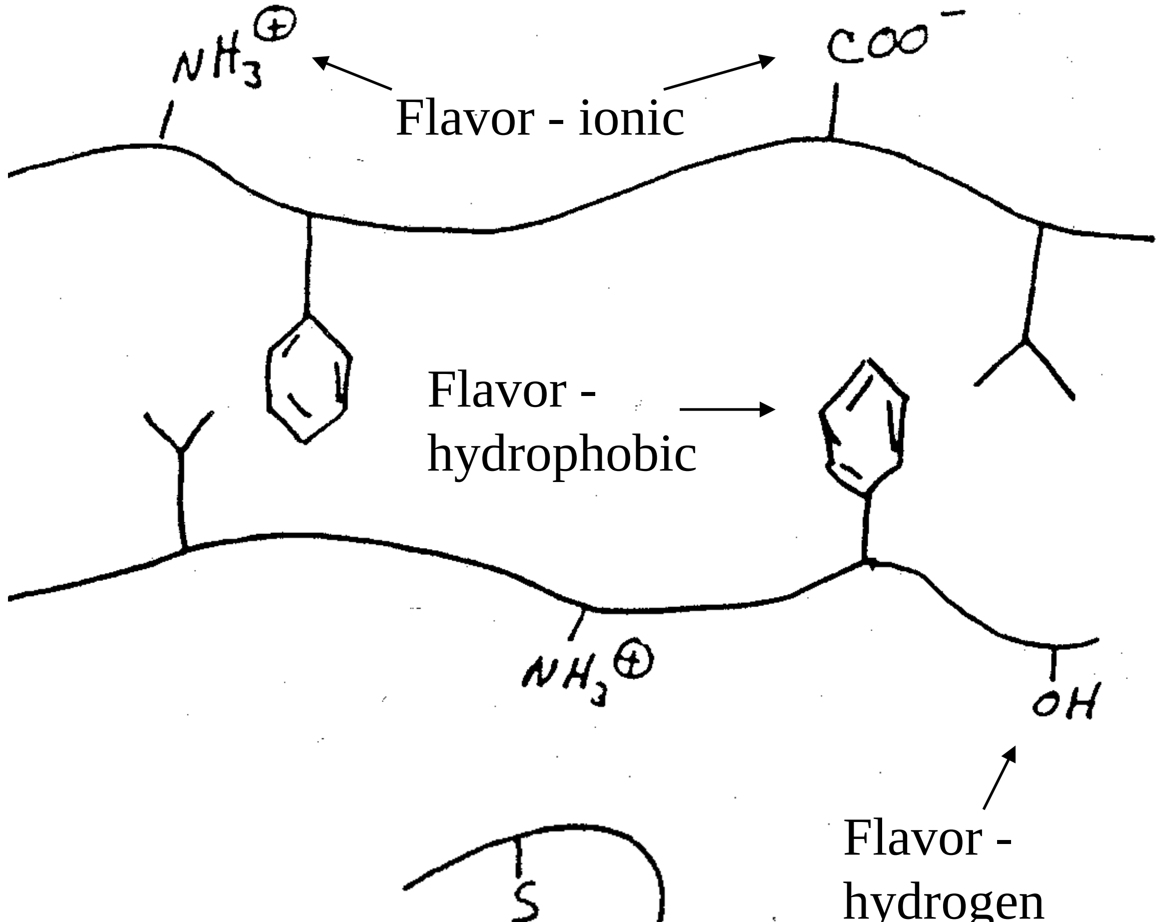
Proteins

- Made up of amino acids – very reactive compounds
- Flavors – made up of very complex, equally reactive compounds
- THINGS HAPPEN!

Flavorings

- One compound may carry the primary flavor character (Character impact compound) but another 20-50 may make up the complete flavor
- Complex systems! We depend upon a delicate balance in compounds and quantities

apple	*n*-hexanal
	trans-2-hexenal
asparagus	dimethyl sulfide
banana	isoamyl acetate
bergamot	linalyl acetate
blueberry	isobutyl 2-buteneoate
blue cheese	2-heptanone
	2-nonanone
bramble (artic)	2,5-dimethyl-4-methoxy-3(2*H*)furanone
butter	diacetyl
caramel	2,5-dimethyl-4-hydroxy-3(2*H*)furanone
caraway	D-carvone
cassia	cinnamic aldehyde
	methoxycinnamic aldehyde
celery	3-propylidene-1(3*H*)-isobenzofuranone
	cis-3-hexenyl pyruvate
cherry	benzaldehyde
	tolyl aldehyde
	benzyl acetate

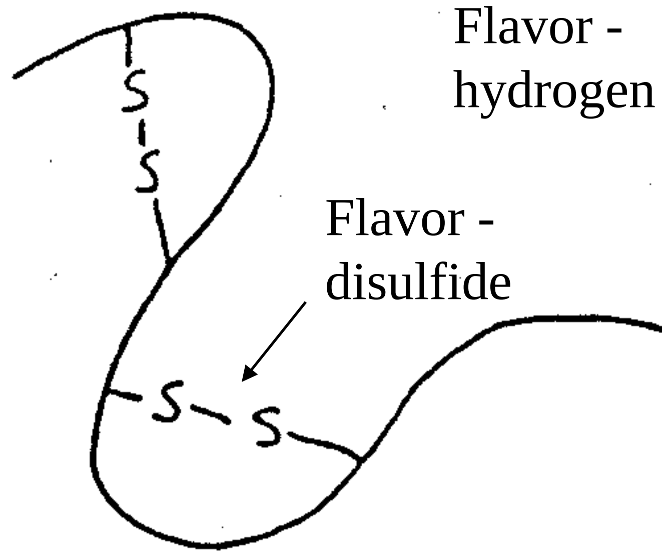


Hydrophobic

Disulfide

2 R-SH ⟶ R-S-S-R

sulfhydryls disulfide



Current understanding of protein-flavor binding mechanisms

Type of interactions	Secondary molecular interactions	Regions or groups of proteins involved	Reversibility	Example of flavours
Physicochemical interactions	Hydrophobic interactions	Interior hydrophobic area of proteins	Reversible	Ketones[1, 2, 3,] aldehydes[2], alcohols[4], ester[5, 6]
	Hydrogen bonds	-OH, -COOH, -SH	Reversible	Aliphatic alcohols[4, 7], lactone[8,] volatile acids[8]
	Ionic bonds/ electrostatic linkages	$-NH_2$, -OH	Reversible	Volatile acid[8, 9]
	van der Waals forces		Reversible	Hydrocarbons [7, 10]
Chemical bondings	Covalent linkages	-S-S-, -SH,$-NH_2$	Irreversible	Aldehydes[11], vanillin[12], sulphur containing flavours[13]

- Wang, K. 2015 Evaluation of protein-flavor binding on flavor delivery and n\and protein-thermal gelation properties in regards to selected Plant proteins. PhD thesis , University of Manitoba, Winnipeg, Manitoba

Sulfur containing flavor RXN

Sulfhydryl groups of cysteine and disulfide groups of cystine in the protein may be involved in these redox rxns.

$$\text{R-S-S-R' + Protein – SH} \longrightarrow \text{R-SH + Protein-S-S-R'}$$

- Volatile disulfides flavors in meat interact with proteins in aqueous solution, resulting in losses of the disulfide aroma comps and formation of the corresponding aroma active thiols."

- (Nobrega, I. C. C.; Mottram, D. S. The formation of meaty aroma compounds in cysteine model systems containing ribose 5-phosphate or ribose. In *Frontiers of Flavour Science*; Schieberle, P., Engel, K. H., Eds.; DFA: Garching, Germany, 2000; pp 487- 491

Reactions/Interactions

Non-covalent binding e.g.

Putrascine, cadaverine, spermadine, and

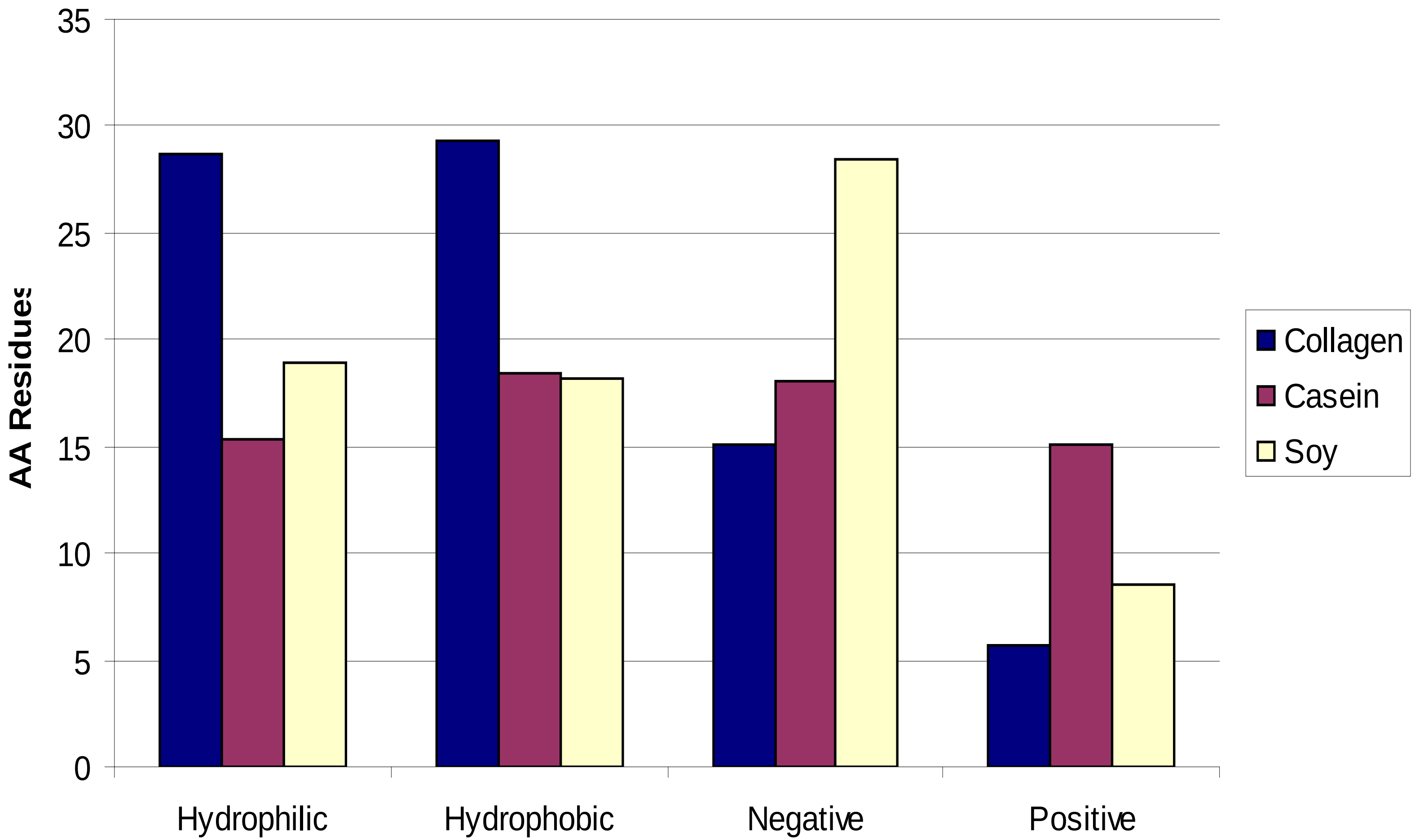
spermine have sensory thresholds 2-5x

Higher in 2% soy protein vs. pure water.

Binding/interaction related to the type of protein

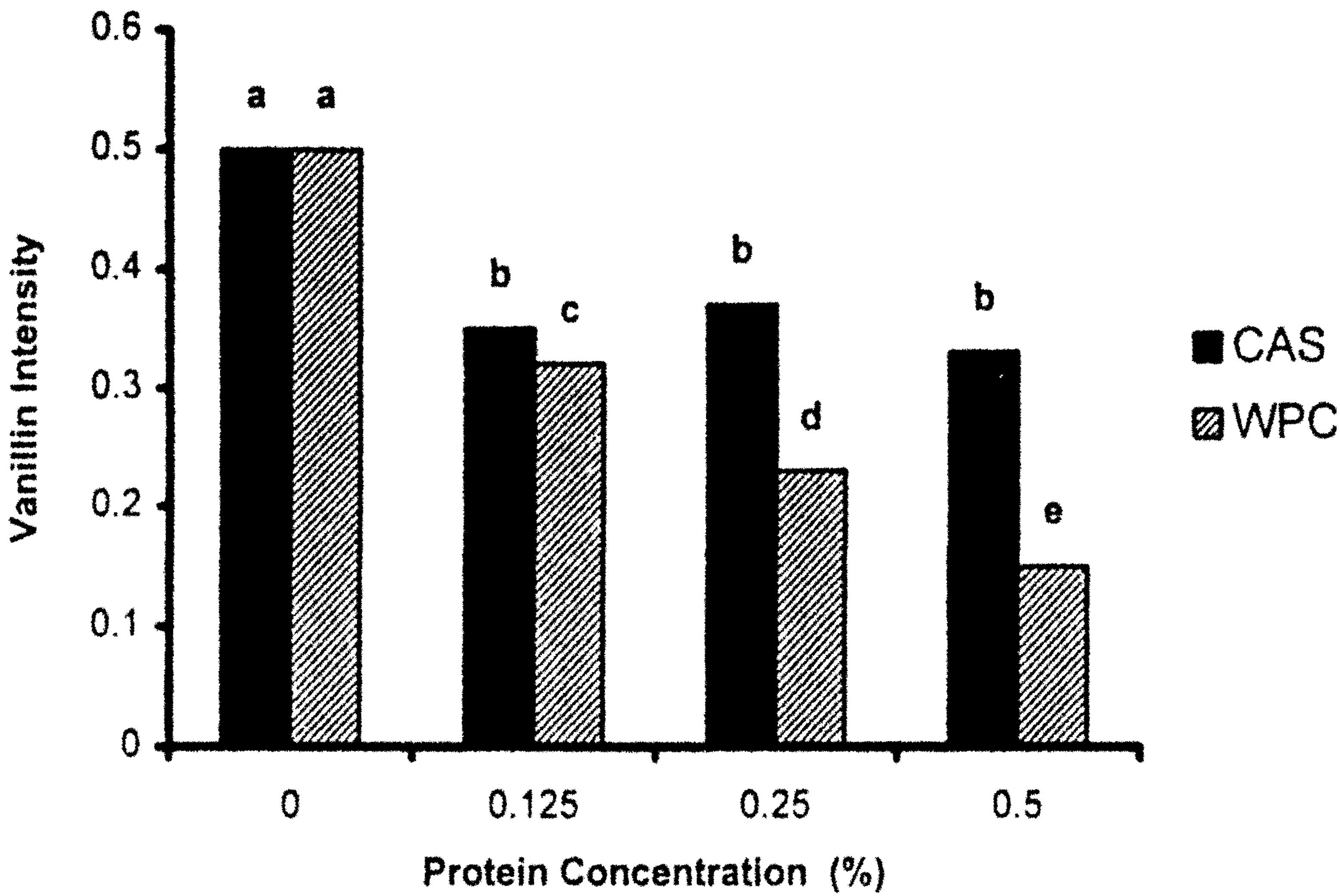
- Soy > Gelatin > Ovalbumin > Casein > Corn

Amino Acid profile differs with Protein



Wang, K 2015

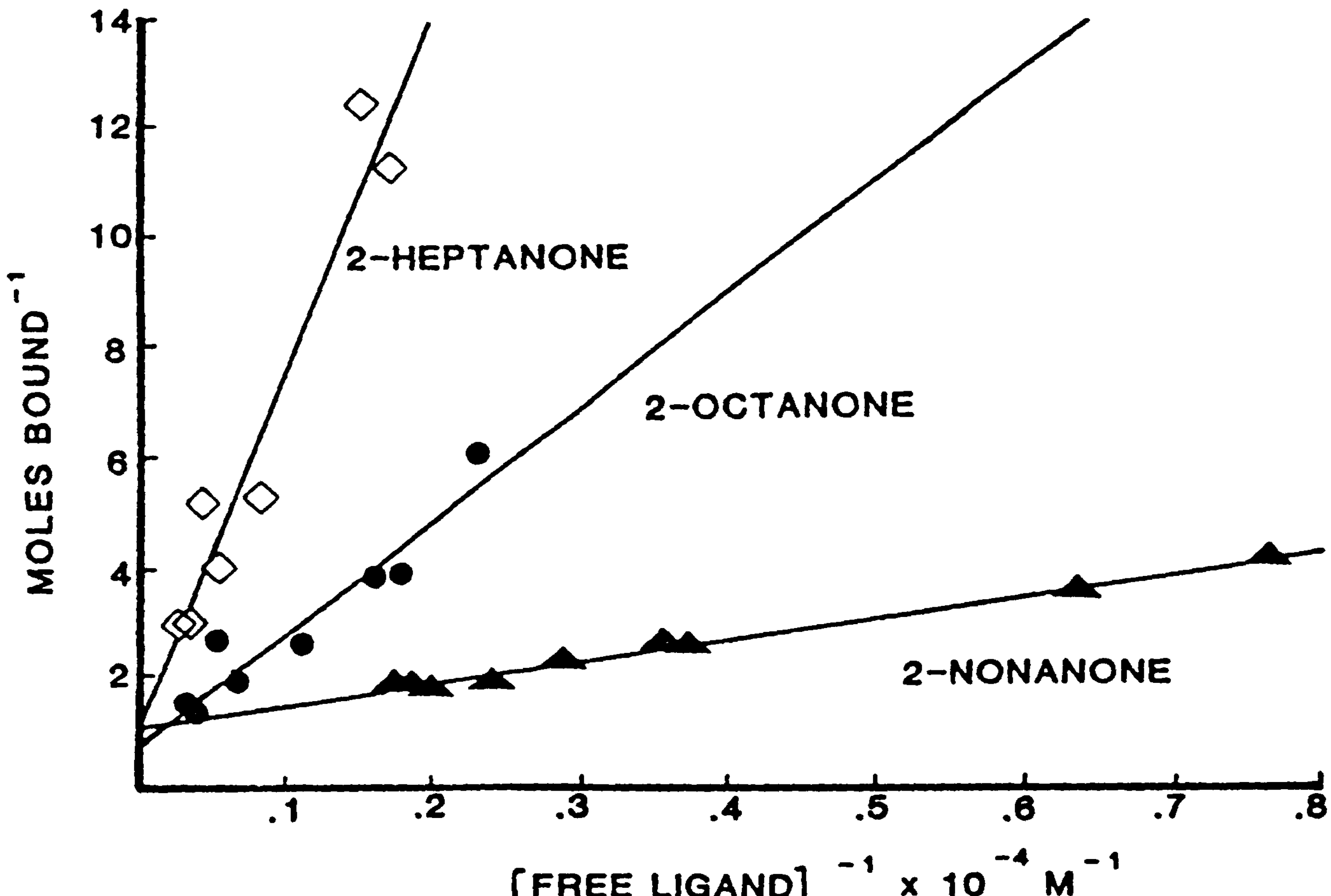
Vanillin binding to dairy proteins



Hansen, A.P.; Booker, D.C. 1996. In: Flavor-Food Interactions. ACS Sym Series 633. ACS

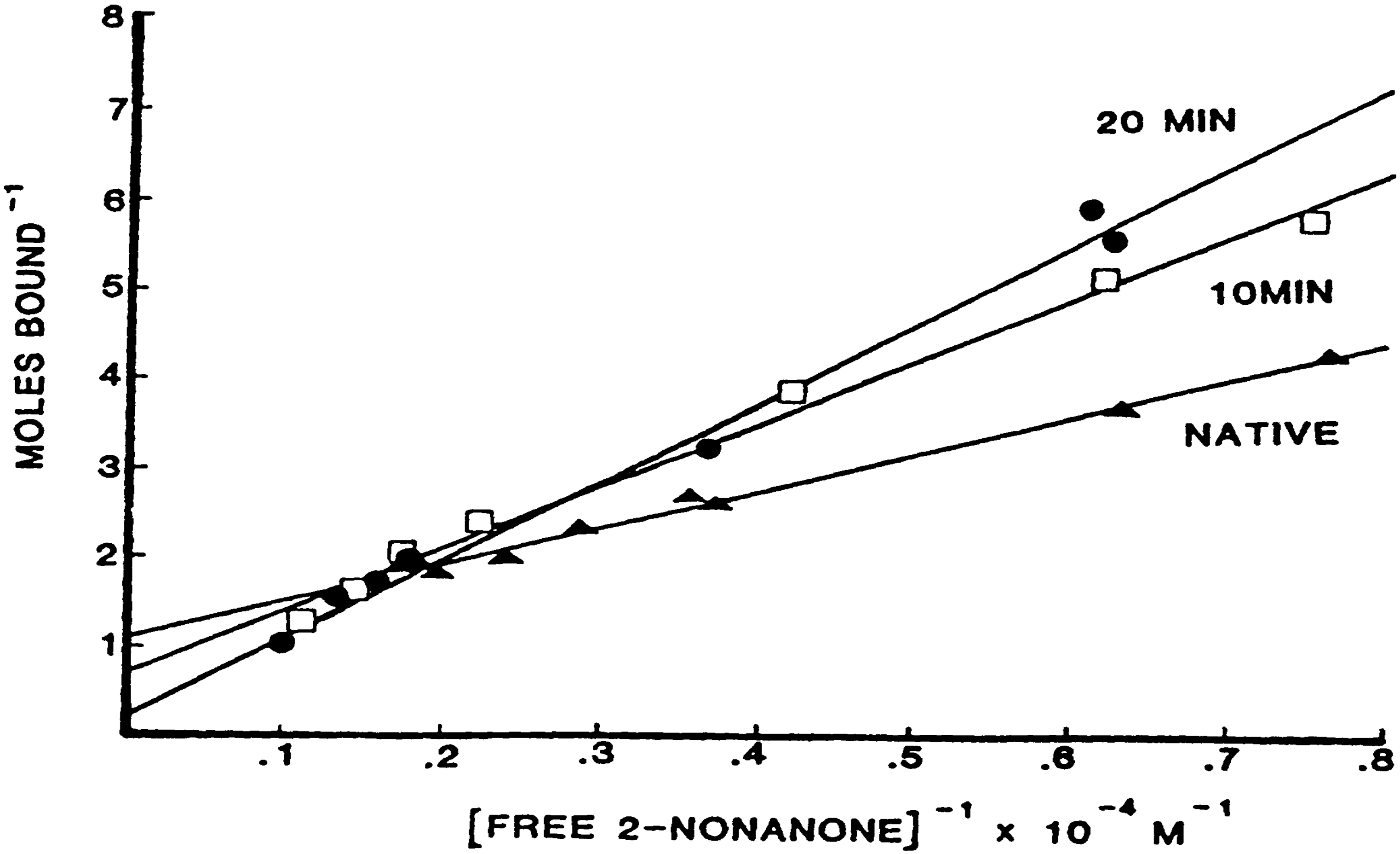
Flavor compound dependent

O'Neill, T.E. 1996. In: Flavor-Food Interactions. ACS Sym Series 633. ACS



O'Neill , T. E. in *Flavor-Food Interactions,* McGorrin, R.J. and Leland, J. V. ACS Sym. Series #633, 1996, 59-74.

Thermal history of protein influences binding (Degree of denaturation)



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Denaturation

Process in which the spatial arrangement of a protein is changed from a native conformation to a more disordered arrangement.

Will affect structure and therefore binding/ interactions.

Food proteins differ widely in their susceptibility to denaturing agents. Some proteins do not denature with ordinary treatments.

Denaturing agents include:

1. Heat
2. Strong acid/alkali
3. Agitation (mixing, foaming)
4. Solvents

What proteins may be “easier” to flavor?

- Chose to spike protein “bars” with selected aroma compounds and determine their binding (loss)
- Proteins studied: Rice, whey, pea and soy
- Eight aroma compounds that characterize various flavorings (or have service as a model compound)
- Measure loss during storage (45C) for 4 weeks

Bar formulation (model)

Ingredient	Percentage by weight (%)
Protein Isolate (Whey, Soy, Pea or Rice)	47.0%
Isomalto-oligosaccharide syrup	33.0%
Triacetin	9.9%
Water	9.0%
Potassium Sorbate	1.0%
Flavor Compound Mixture	0.1%

Sia and Reineccius, 2017 unpublished work. Univ of Minn, St Paul

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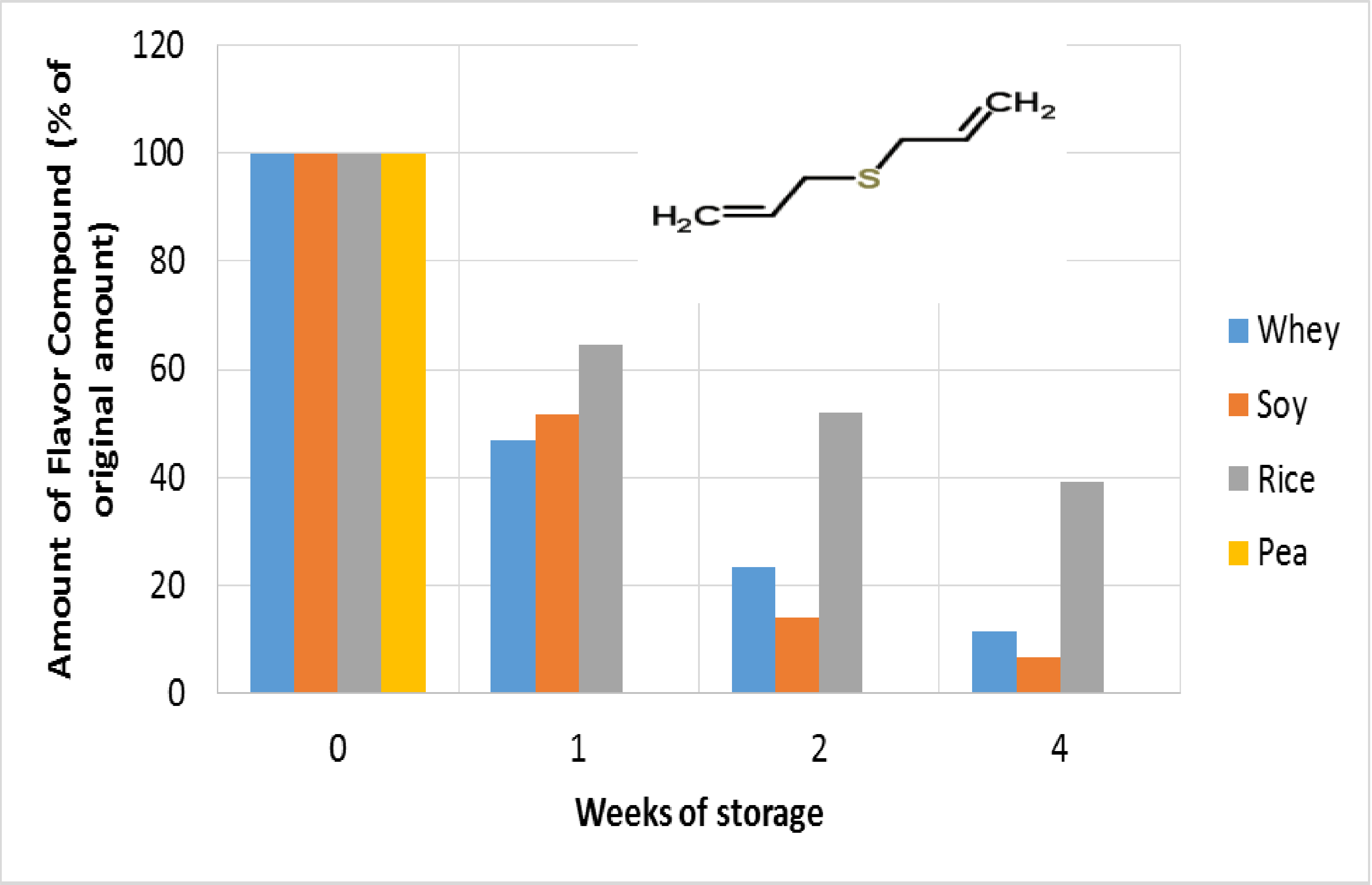
Loss of allyl sulfide due to reaction with proteins – 45C storage



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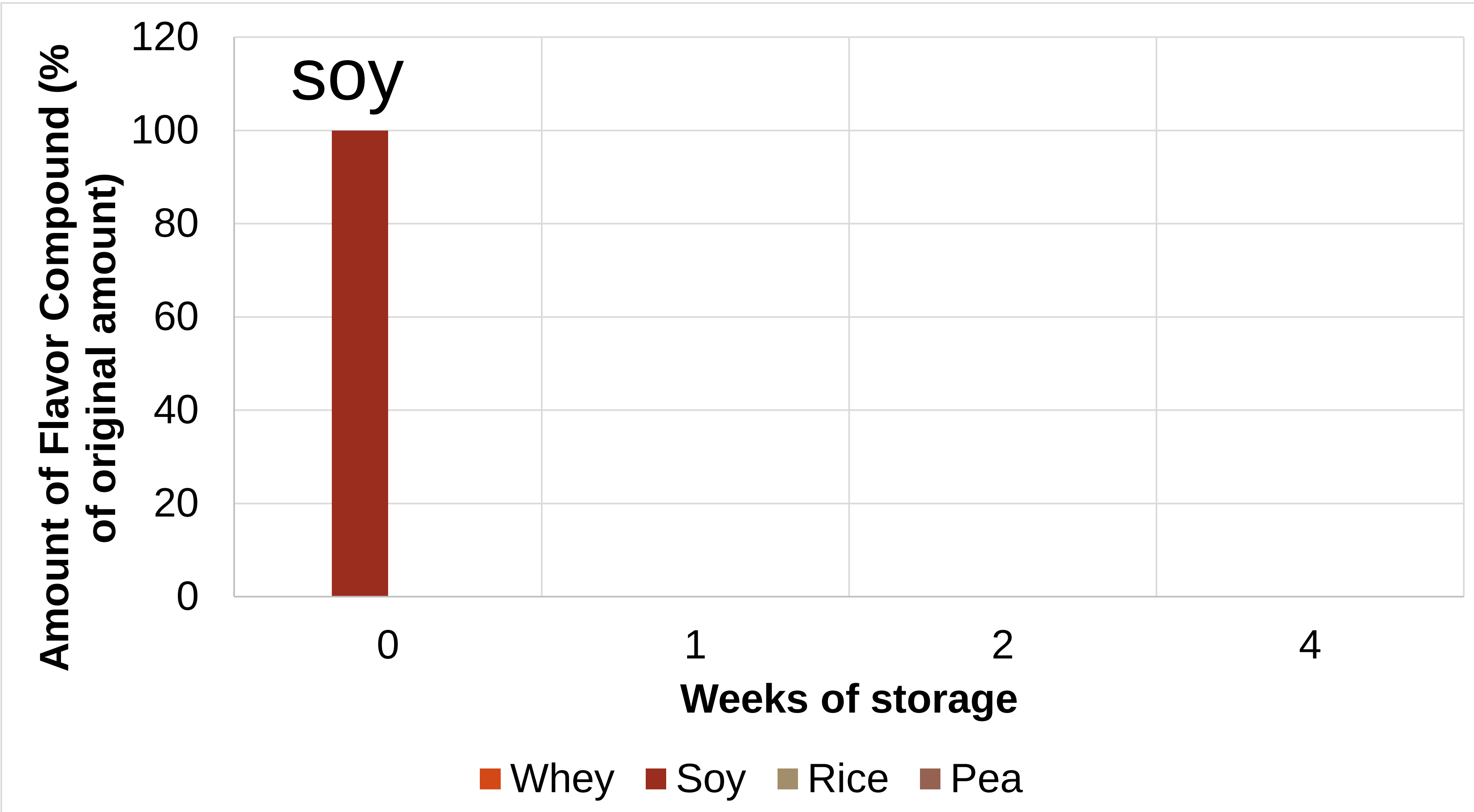
Furfuryl mercaptan – roast and ground coffee aroma



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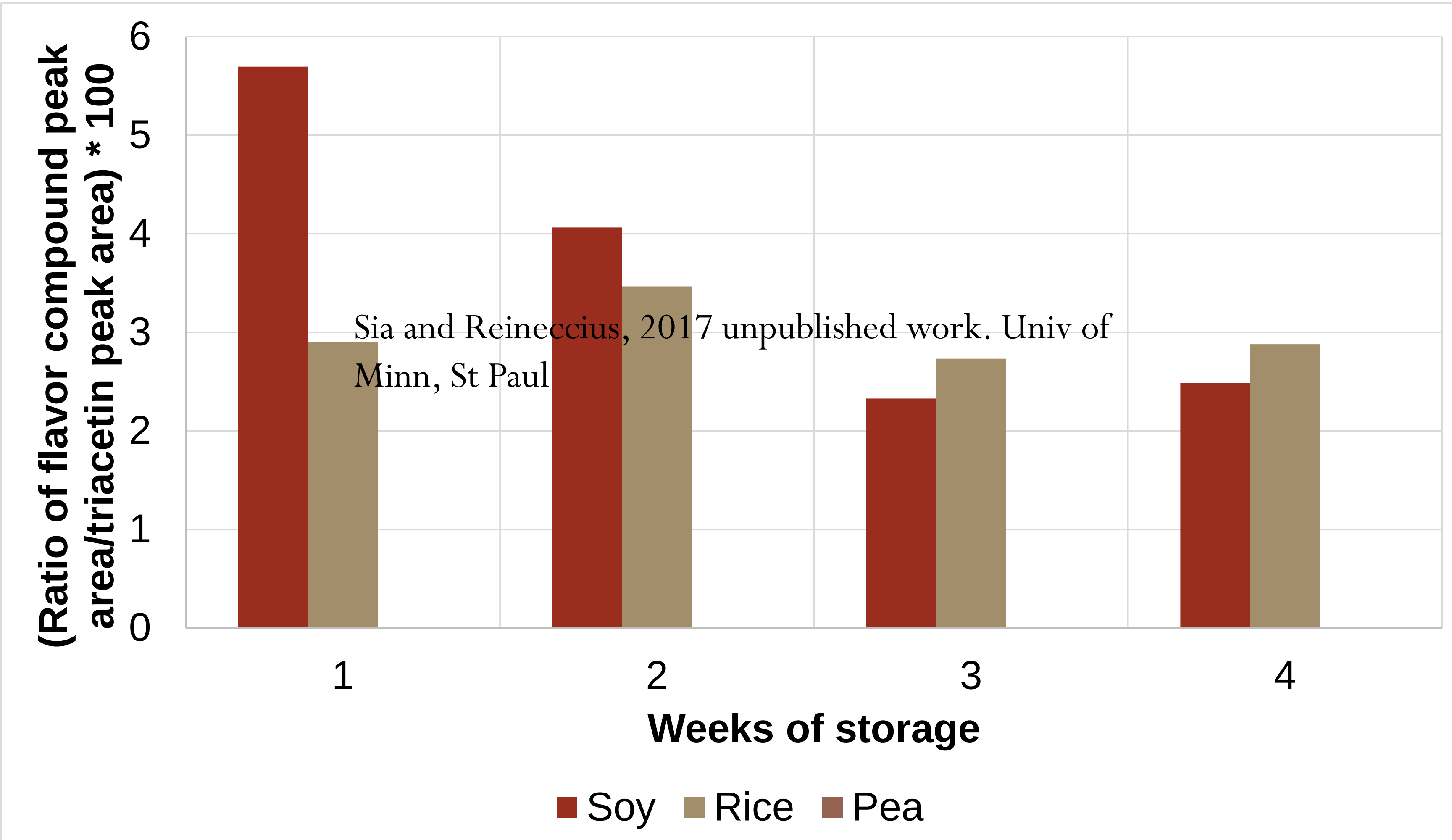
Benzaldehyde - Cherry/almond



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Protein hydrolysates

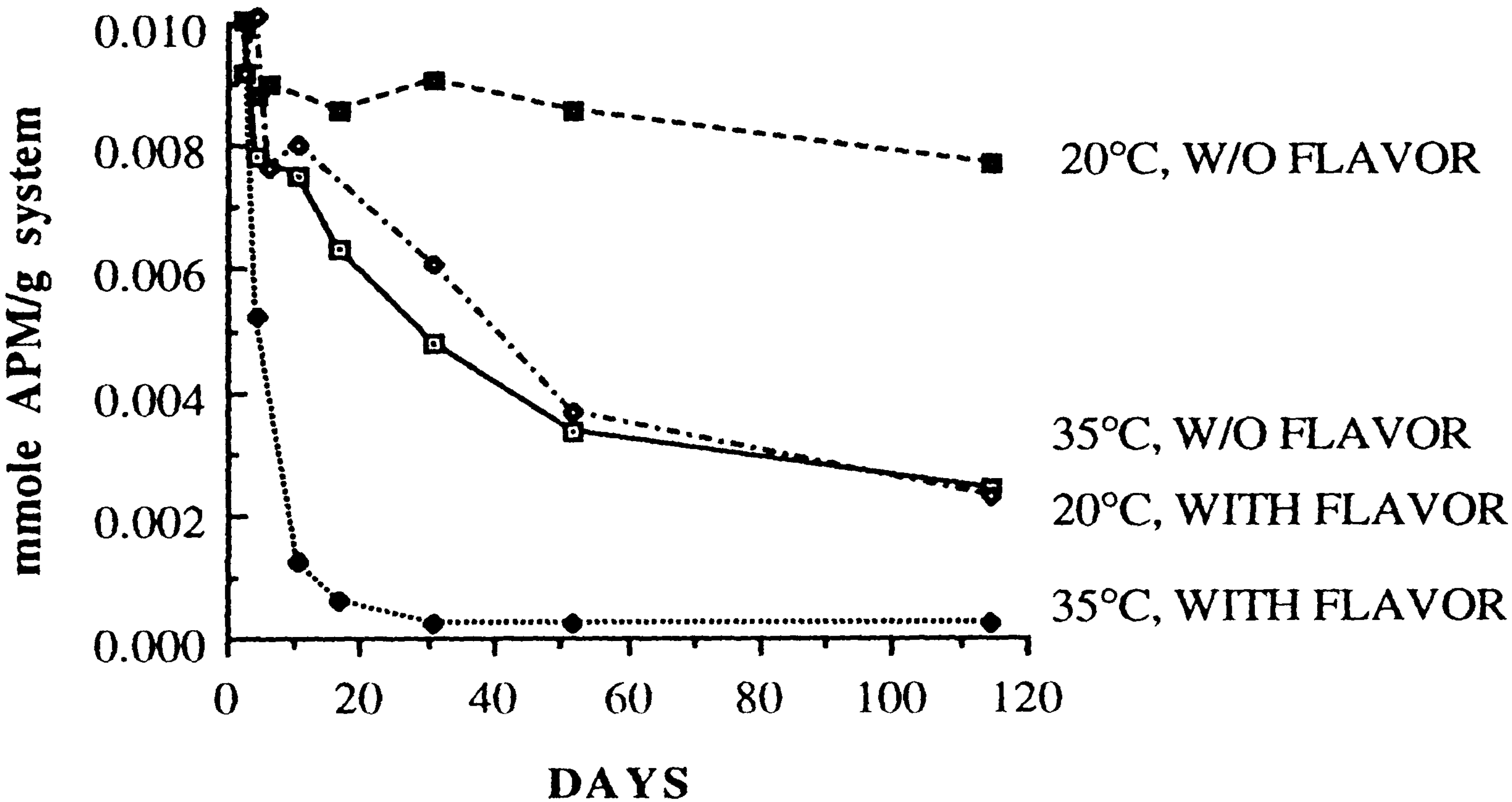
- The greater the degree of hydrolysis, the less the “interaction”

- That does not mean they have no influence on flavor stability - free amino acids are reactive and can function as catalysts

Example - Maillard reaction/Schiff Base

- Aldehydes react with proteins/amines
- Irreversible reactions

Loss of APM in chewing gum due to reaction with flavoring



Schirle-Keller, J.P.; Reineccius, G.A.; Hatchwell, L.C. 1996. In: Flavor-Food Interactions. ACS Sym Series 633. ACS

Solutions?

- You probably cannot change the food composition
  - Maybe protein source?
  - Humectant (if one is used – PG forms acetals)
  - Think about options to remove/replace materials that react with flavorings
- Dry products – water activity influences chemical reactions (but changes food also)

Solutions (my choice)

- 1. Select the right flavor – think about key compounds that characterize each flavor. Anything depending upon aldehydes or sulfur compounds for key character are going to be problematic.

- 2. For dry products, encapsulation of the flavoring.
  - Separates the flavor from the food until flavor is released.

  (Release is typically achieved by water (dissolution)
  - Aw during storage/prior to rehydration is important (explain)
  - Spray dry formulation must be adjusted to perform in this application (explain)

Protein/flavor interaction summary

- Chemical effects - may change both (intensity and character)
  - Binding and release (?) - hydrophobic, disulfide bonds, ionic bonds
  - Irreversible effects - Maillard reaction or other catalyzed reactions

Chemical effects change with:

- Protein type (amino acid composition and tertiary conformation)
- Thermal processing of protein (denaturation)
- Hydrolysis
- Flavor compound
- Environment (pH, redox potential, other ingredients, etc.)

Bottom line - what do we know?

- Unfortunately, not as much as well need to know
- We know to expect changes (qualitative) but we do not have quantitative knowledge.

My approach?

- Work with those that have experience with flavoring high protein products learn how to best manage.

- Challenge anyone who says they know all.

This still an art!

Thank you and ….. questions?