Protein + Flavor = A Formulation Challenge

Robert J. McGorrin, Ph.D., CFS
Department Head and Jacobs-Root Professor
Food Science & Technology
Robert.mcgorrin@oregonstate.edu

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Overview

- A basic understanding of flavor quality and experience

- Flavor stability, interactions, and causes of flavor changes

- Flavor challenges with using proteins

- Recommendations for protein flavor applications

- Flavor formulation and off-flavor masking examples
Food Products are Complex Systems!

- Water
- Fat
- Protein
- Carbohydrates
- Minerals
- Emulsifiers
- Gums
- Antioxidants
- Vitamins
- Phytonutrients / botanicals
- Color
- Flavor
The Significance of Flavor

- Commercial success of a newly launched food product is directly linked to flavor.
- Flavor quality is a major driver of consumer acceptance for food products.
- Desirable flavor sensory attributes: Rapid impact, balanced/rounded, lacking off-flavors, quick clear-out, minimal aftertaste.
What is Flavor?

The **Flavor Experience** = **Aroma** + **Taste** + **Chemesthesis**

- **Aroma**: Aromatics
  - Volatile
  - Primarily fat soluble
  - Over 8,000 known aroma chemicals
  - Organic (carbon) molecules with oxygen, nitrogen, sulfur
  - Perceived ortho-nasal (smell) and retro-nasal (mouth)

- **Taste**: Tastants
  - Non-volatile
  - Water soluble (saliva)
  - Sweet, sour, salty, bitter, umami

- **Chemical Feeling**: Chemesthesis (Trigeminal nerve)
  - Skin response to chemical irritation; not only in mouth
  - Examples: Pepper *burn*, menthol *cooling*, cranberry *astringency*
Chemistry of Flavors

Volatile compounds (Aromatics)

- Typical molecular weight range between 34 – 300
- Boiling points:
  - -60°C Hydrogen sulfide (egg)
  - 20°C Acetaldehyde (orange juice)
  - 131°C Hexanal (green; rancid)
  - 320°C δ-Dodecanal (coconut; cream)
- A natural flavor can contain 200 – 1,000 volatile constituents
- Individual components are typically present at parts-per-million to parts-per trillion. Trace levels/high impact!
- Some aroma chemicals provide unique flavor characters or sensory impressions (so-called “character-impact compounds”)
Chemistry of Flavors

Examples of volatile Character-Impact compounds

Benzaldehyde
cherry, almond

Methyl anthranilate
Concord grape

Menthol
peppermint

Nootkatone
grapefruit

2-Methoxy-3-isobutyl pyrazine
green pepper

Vanillin
vanilla
Chemistry of Flavors

Non-volatile compounds (Tastants)

- Typical molecular weight range between 40 – 1,000
- Sweet: sucrose, fructose, aspartame, sucralose
- Bitter: caffeine, quinine
- Salty: sodium chloride, potassium chloride
- Sour: citric acid (citrus sour), butyric acid, lactic acid (dairy); acetic acid (vinegar)
- Savory: monosodium glutamate, amino acids, HVP’s
Causes of Flavor Degradation

- Heating
  - High temperature processing
  - Volatile flash-off
- pH
- Metal ions
  - Iron
  - Copper
- Lipid oxidation – Air / light
- Enzymes
  - Lipoxygenase + fatty acids in soybean oil
- Maillard reactions
- Interactions of flavors with food ingredients
  - Vanilla flavor reacts with whey protein concentrate
  - Vitamin reactions / breakdown
Flavors and Proteins

The addition of protein to a food product may alter flavor by:

1. Imparting undesirable off-flavors
   "Beany" flavors; bitter tastes; astringent mouthfeel

2. Changing the food’s flavor profile due to:
   - Flavor interactions
   - Flavor binding
   - Flavor release

Depending on the specific protein, and how they interact with it, flavors come across as either “brighter” or “muted”. We’re just beginning to understand the chemistry behind the flavor changes
Flavor Changes from Proteins

1. Imparting undesirable off-flavors

- Proteins generally should not impart flavor characteristics or contribute flavor
  
  However . . .

- Typical ingredient processing and storage conditions can produce undesirable off-flavors:
  - Volatile compounds produced from amino acids or protein fragments
  - Oxidation of trace amounts of fat
  - Maillard browning reactions
Flavor Changes from Proteins

1. Imparting undesirable off-flavors

- Soy protein
  - Beany, green, cereal, bitter
- Pea protein
  - Earthy, grassy, nutty, savory; grainy mouthfeel
- Rice (brown) protein
  - Rancid, smoky
- Whey protein concentrate (WPC)
  - Grassy, hay, cheesy, astringent
- Whey protein isolate (WPI)
  - Cardboard, wet dog, cooked milk, cabbage, bitter, astringent
- Casein (milk protein)
  - Stale milk, gluey, cheesy, musty, sour
- Protein hydrolysates
  - Astringency
## Protein Off-Flavors

200 volatile chemical compounds have been identified in whey (dry and liquid) that may influence/contribute to their flavor and aroma in finished product.

### Fatty acids
- Acetic acid **Vinegar**
- Hexanoic acid **Sweaty**
- Butanoic acid **Cheesy/rancid**

### Amino Acid breakdown
- Cysteine, methionine, tryptophan, phenylalanine
- Dimethyl sulfide **Garlic/rubbery**
- Dimethyl trisulfide **Cabbage**
- o-Aminoacetophenone **Grape**
- Methional **Potato**
- 2-Methoxy phenol **Smoky**

Adapted from: Carunchia Whetstine, Croissant, Drake (2005).
Protein Off-Flavors

Fat oxidation

<table>
<thead>
<tr>
<th>Fat</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexanal</td>
<td>Green grass</td>
</tr>
<tr>
<td>Nonanal</td>
<td>Fatty/citrus</td>
</tr>
<tr>
<td>Octanal</td>
<td>Citrus/green</td>
</tr>
<tr>
<td>Decanal</td>
<td>Fatty</td>
</tr>
<tr>
<td>(E)-2-Nonenal</td>
<td>Cucumber/old books</td>
</tr>
<tr>
<td>(E,Z)-2,6-Nonadienal</td>
<td>Cucumber</td>
</tr>
<tr>
<td>(E,E)-2,4-Decadienal</td>
<td>Fatty/oxidized</td>
</tr>
<tr>
<td>γ-Nonalactone</td>
<td>Coconut</td>
</tr>
</tbody>
</table>

Maillard reactions

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Methyl-3-furanthiol</td>
<td>Brothy/burnt</td>
</tr>
<tr>
<td>2-Acetyl-1-pyrroline</td>
<td>Popcorn</td>
</tr>
<tr>
<td>3-Hydroxy-4,5-dimethyl-2(5H) furanone</td>
<td>Maple/spicy</td>
</tr>
<tr>
<td>2,5-Dimethyl-4-hydroxy-3(2H) furanone</td>
<td>Burnt sugar</td>
</tr>
</tbody>
</table>

Adapted from: Carunchia Whetstine, Croissant, Drake (2005).
Maillard Reactions and Flavor

*Maillard Reactions / Thermally-processed foods:*
- Roasted peanuts
- Toasted bread
- Fried chicken
- Baked potatoes
- Grilled flavors
The Maillard Reaction

Reducing Sugars + Amino Acids → “Brown” aromas and colors

Early Maillard Reaction
- Amadori Rearrangement

Advanced Maillard Reaction
- Fission: Dicarbonyls
- Dehydration: Dicarbonyls, amino cmpds.
- Strecker Degradation: Aldehydes, creatinine

**MELANOIDINS**
(brown polymeric pigments)
Thermally-Generated Flavors

Chemical Processes: Maillard Reactions, Caramelization, Strecker degradation

COOKING OF FOODS

Positive Flavors
- Roasted peanuts
- Baked potatoes
- Toasted bread
- Grilled steak
- Fried chicken

FOOD STORAGE
(Staling over shelf-life)

Flavor Defects
- Dried / UHT milk
- Whey powder
- Dried sour cream
- Cheese powder
- Soy milk
Maillard Reaction Parameters

- Heating Temperature
- Heating Time
- pH
- Water Activity
Rate Influence by Temperature

pH Effects

Reaction with D-Glucose

Absorbance, 420 nm

pH

L-Lysine
L-Alanine
L-Arginine
Off-Flavor Formation in Proteins

Maillard reaction chemistry

Dried sour cream  Marsili, ACS Symp. Ser. 971, 2007
Cheese powder  Marsili, ACS Symp. Ser. 971, 2007
Thermally Generated Off-Flavors

**SPRAY-DRIED CREAM**

Diacetyl + Arginine $\xrightarrow{\text{Maillard Rxn.}}$ 2,4,5-Trimethylthiazole ("melon", "ripe kiwi")


**SPRAY-DRIED MILK POWDER**

Tryptophane $\xrightarrow{\text{Maillard Rxn.}}$ Benzothiazole ("sulfuric, quinoline") + 2-Aminoacetophenone ("musty, stale")

M. Preininger and F. Ullrich in *Gas Chromatography-Olfactometry*, ACS Symp Series 782, 2001, p. 46.
Thermally Generated Off-Flavors

**ULTRA-HIGH TEMPERATURE (UHT) MILK**

Dicarbonyls + Amino acids $\xrightarrow{\text{Maillard Reaction}}$ 2,6-Dimethylpyrazine

$\xrightarrow{\text{Strecker Degradation}}$ 2-Ethyl-3-methylpyrazine

$\xrightarrow{}$ 2-Ethylpyrazine

$\xrightarrow{}$ Methional


2. Changing the food’s flavor profile due to:

- Flavor interactions
- Flavor binding
- Flavor release

- Flavor perception in food systems is governed by complex multiple interactions with proteins, as well as carbohydrate and fat components.

- Food systems contain multiple phases and structures which can substantially influence flavor interactions:
  - Phases: Emulsions, dispersions
  - Structures: Membranes, interfaces

- The relative balance of different flavor-ingredient combinations ultimately influences overall flavor perception.

Flavor Interactions with Proteins

Definitions of some flavor interaction terms:

- **Flavor Absorption**
  - Trapping of volatile flavor compounds onto non-volatile food constituents (e.g., proteins)

- **Flavor Binding**
  - Covalent bond formation; hydrogen bonding; or hydrophobic interactions between flavor and protein

- **Flavor Release**
  - **Aroma**
    - Availability of aroma compounds to be freed from the bulk of the food into the gas phase for sensory perception
  - **Taste**
    - Availability of non-volatile compounds to be freed from the bulk of the food into the aqueous phase for sensory perception

http://chubbylemonsscience.blogspot.com
Protein-Flavor Interactions

- Proteins in food can interact with flavor compounds
- Flavor–protein binding interactions: The most studied are the binding of flavors to soy protein and casein (milk protein)
- Flavor binding – retention or absorption of volatiles onto non-volatile protein
- Forms of interactions
  - Hydrogen bonding: oxygen, nitrogen, sulfur
    reversible
  - Covalent bond formation irreversible

Flavor Interactions with Proteins

Food Protein (α-Helix) + Flavor Chemical Mixture → Protein-Flavor Complex
Protein-Flavor Interactions

- In general, alcohols and ketone-containing flavors reversibly bind through hydrophobic interactions and hydrogen bonding.

- **Aldehyde flavors** may chemically react with amino groups of proteins, forming irreversible covalent bonds (Schiff bases).

- Binding capacity depends on pH, temperature, moisture content, water activity, salt level, **degree of denaturation**. *Protein denaturation can increase flavor absorption, through greater exposure of hydrophobic regions.*

- Result: 1) **Flavor fade** (reduction of flavor intensity)
  2) **Flavor imbalance** (due to selective binding)
Flavor-Food Interactions:  Flavor Imbalance

Reactions:  Flavors + Amino Groups

Benzaldehyde (Cherry) + Aspartame

Neotame

“Schiff base”

Flavor Binding and Protein Structure

- Protein binding properties are influenced by its 3-D structure
- Hydrogen bonds between amino acids
- Disulfide bridges between amino acids
- Hydrophobic “pockets”
- Ionic complexes

[Link to the Chemguide website for more information]
Flavor Binding and Protein Structure

Disulfide bond formation

Sulfur bridge formed

Note: Sulfur flavors (mercaptans, thiols, etc.) also form disulfide bonds with proteins
Sulfur Amino Acids – Off-Flavor Contributors

Methionine

Cysteine

Cystine
Flavor Binding and Protein Structure

Hydrophobic pockets

Ionic regions
Flavor Binding/Interaction Related to the Type of Protein

Soy > Whey > Gelatin > Casein > Corn

- Inherent tightly-bound off-flavors in soy (SPC, SPI), whey (WPC, WPI), casein
- Need to be de-flavored by protein hydrolysis or membrane processing

Vanilla Binding with Dairy Proteins

**Flavor-Protein Interactions:** *Flavor Fade*

Hansen and Booker in *Flavor-Food Interactions*, McGorrin, R.J. and Leland, J. V.
ACS Sym Series #633, 1996, 75-89.
Flavor-Protein Interactions: Flavor Fade

Vanilla Binding with Dairy Proteins

![Graph showing the binding of vanilla with different proteins over time.]

Flavor-Protein Interactions:  Protein Denaturation

Effect of Heat Treatment (75°C)  
2-Nonanone Binding with Whey Protein

O’Neill, T. E. in  Flavor-Food Interactions, McGorrin, R.J. and Leland, J. V.  
Protein - Flavor Applications
Flavor Challenges
High-Protein Foods & Beverages

- Difficult to select/choose appropriate flavors
- Challenges to control the proper level of flavoring
- Challenges to achieve the desired flavor intensity in the finished product
- Continued opportunity for taste improvement in nutritional food and drink products
Protein Sources 2011—2017 Launches North America Food & Beverages

Mintel GNPD
Competition among Protein Options (2011 – 2017)
Challenges with Flavoring High-Protein Foods

• Flavors are challenged by adding nutritional ingredients! Proteins (Soy, whey, casein, pea, rice) + HTST, UHT (Burnt, caramelized, nutty, beany, sulfuric, bitter) Amino acids, minerals (Bitterness, metallic off-flavors)

• Manufacturers use many combinations / blends of proteins
  ➢ Soy, whey
  ➢ Soy, whey, caseinate, rice
  ➢ Whey, pea, rice

• To achieve optimum protein value / PDCAAS / PER
• Concentrates, isolates, hydrolyzed
• Minimize inherent off-flavor characteristics of an individual protein
  • Soy and whey proteins complement each other
  • Soy manages sulfide and eggy notes from whey

• Net: A fairly complicated process from a flavorist’s perspective
Flavor Development - Proteins

• Need to use flavor by the “bucket-load” (4-10 X more)

• Proteins are good at binding / absorbing flavor

• Proteins contribute: Bitterness, astringency, chalkiness (particularly true if beverage is acidic, pH 3.5)

• Optimum pH 6-7 to avoid gritty texture / astringent taste

• However, pH 3.5 works best with citrus flavors (orange, lemon); actually enhances flavor; makes flavors “pop”
Flavor Development - Proteins

Hydrolyzed proteins:
• Clear beverages – flavor issues
• Hydrolyzed proteins contribute off-tastes
  • Sulfur amino acids: Rubbery (cysteine, methionine)
  • Flavors not muted as much as intact proteins; less binding, so don’t need to add as much flavor

Flavor Rebalancing:
• Added flavor is initially unbalanced; (need to wait 5 days before evaluating)
• Formulate flavor to increase top notes, middle notes
• Compensates for expected losses during shelf-life, retort heating, etc.
• Will be balanced in finished product
Challenges with Flavoring High-Protein Foods

**Protein Bars**
- Low moisture  $a_w = 0.2$
- Non-thermal process
- Flavor system is “immobile”
- RT shelf life temperature swings

**Protein Beverages**
- High moisture  $a_w = 1.0$
- Thermal process
- Flavors more reactive
- Flavor scalping
- Refrigerated
Fastest-Growing Flavors (2018)

Protein Beverages, Dairy

Chocolate          36%
Unflavored/Plain   33%
Vanilla (Nat.)     17%
Strawberry         14%

Protein Savory

Chicken           61%
Cheese            39%

Mintel GNPD 1Q, 2Q 2018  389 SKU’s in U.S.A. and Canada
## Appropriate Flavor Types

<table>
<thead>
<tr>
<th>Protein Bars</th>
<th>Protein Beverages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Browned / roasted Flavors</strong></td>
<td></td>
</tr>
<tr>
<td>Chocolate</td>
<td></td>
</tr>
<tr>
<td>Double fudge</td>
<td></td>
</tr>
<tr>
<td>Mocha/coffee</td>
<td></td>
</tr>
<tr>
<td>Chocolate/peanut butter</td>
<td></td>
</tr>
<tr>
<td>Caramel/peanut</td>
<td></td>
</tr>
<tr>
<td>Cookies &amp; creme</td>
<td></td>
</tr>
<tr>
<td><strong>Challenges</strong></td>
<td></td>
</tr>
<tr>
<td>Vanilla</td>
<td></td>
</tr>
<tr>
<td>Strawberry</td>
<td></td>
</tr>
<tr>
<td>Citrus flavors</td>
<td></td>
</tr>
<tr>
<td><strong>Chocolate; Fruit flavors</strong></td>
<td></td>
</tr>
<tr>
<td>Chocolate</td>
<td></td>
</tr>
<tr>
<td>Banana creme</td>
<td></td>
</tr>
<tr>
<td>Peach mango</td>
<td></td>
</tr>
<tr>
<td>Cookies &amp; crème</td>
<td></td>
</tr>
<tr>
<td>Butter pecan</td>
<td></td>
</tr>
</tbody>
</table>
Flavor Suppliers

• Optimum to involve flavor house early in the process!

• Provide as much information as possible:
  - Moisture content, pH
  - Heat process / upper temperature
  - Room temperature, refrigerated, frozen
  - % protein
  - Vitamins, natural/high-potency sweeteners

• Cuts development time tremendously!
Protein Milk

- Milk protein concentrate
- 25g protein/serving
- Shelf-life: 100 days

Flavors: Chocolate, Vanilla, Strawberry, Cookies n’ Cream
High Protein Frozen Yogurt

- Protein: 20 g
  - WPC, WPI
- Live active probiotics
- Flavors
  - Banana vanilla
  - Dutch chocolate
  - Vanilla bean
  - Blueberry pomegranate
Protein Ice Cream

- Skim and whole milk, whey protein conc.
- 10 g protein/serving
- Flavors:
  - Dutch chocolate
  - Mint chip
  - Blueberry pomegranate
  - Coconut
  - Mocha
  - Vanilla bean
  - Dark chocolate toffee
Protein Bars

Brown rice protein, sacha inchi protein

Protein Gel Shots

Soy protein isolate
Beyond Meat: Chicken-Free Strips

Introduced 2013: “Looks, feels, tastes and acts like chicken – without the cluck”
Beyond Meat: Chicken-Free Strips

Protein
Soy Protein Isolate
Pea Protein Isolate

Flavor system
Chicken flavor (yeast extract)
Hickory smoke
Spices
Plant-Based Analog Meats

1. Beyond Meat Beast Burger 2.0
   Pea Protein Isolate

2. Impossible Burger
   Proteins: Wheat, potato, soy

3. Tofurky Vegetarian Feast
   Soy Protein (Tofu)

February 2017  pp. 26-37
Flavor Masking

Addition of other agents or flavors to “mask” / suppress undesirable off-odors, aftertastes, bitterness

<table>
<thead>
<tr>
<th>Flavor defect</th>
<th>Food product / ingredient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beany, grassy, rancid</td>
<td>Soy beverages, bars</td>
</tr>
<tr>
<td>Harshness, bitterness</td>
<td>Peptides</td>
</tr>
<tr>
<td>Astringency</td>
<td>Low pH, whey protein isolates</td>
</tr>
<tr>
<td>Vit. B1 / thiamin “meaty”</td>
<td>Vitamin fortification</td>
</tr>
<tr>
<td>Metallic, chalky</td>
<td>Mineral, calcium fortification</td>
</tr>
</tbody>
</table>

Flavor Masking

Example #1  Protein Off-flavor

• Flavor Congruency – “Systems approach”
  – Select a flavor system which also contains the inherent off-flavor aspects of a particular protein
    • Example: “Earthly” notes – pea protein; “beany” notes – soy
    • Complement with use of peanut or nut flavors to mask

• Flavor Completion / Insertion
  – Instead of masking undesirable notes, utilize them as part of the flavor system
    • Example: “Green” notes from soy protein
    • Additive effect with “jammy” strawberry flavor that lacks green notes
Flavor Masking

Example #2  Soymilk off-taste

• Taste
  – Soy protein isolates tend to become increasingly bitter as pH is lowered
  – Vanilla and peach flavors are useful to mask bitter off-notes (and the “beany” flavor of soy)
  – Nanoprocessing (nanoshear) may produce creamier taste; flavor emulsion stability
  – Benefit: less flavor is used for same taste effect
Flavor Masking

Example #3  Bitterness off-flavor

• Bitterness is typically modulated by:
  (1) increasing sweetness
  (2) blocking the bitter taste receptors
• Bitterness blockers (“B-blocker”)
  – Sodium chloride
  – Monosodium glutamate
  – Adenosine monophosphate

Flavor Masking

Example #4  Astringency

• Not a flavor, but a mouth drying sensation
• Biggest challenge in whey beverages
• **pH level:** Increasing the pH above pH 3.5 decreases astringency, but heat stability becomes more challenging and clarity decreases.

• **Flavor selection:** Tropical flavors (mango, pineapple, coconut), peach, apple work well with whey protein ingredients; mask whey off-flavor and aroma.

• **Berry and citrus flavors** (strawberry, raspberry, orange) are a challenge to use with whey protein ingredients; do not mask whey flavor and aroma as well as tropicals.

• **Complementary ingredients:** Adding larger carbohydrates such as soluble fiber, gums also may decrease astringency
Flavoring high-protein foods and beverages is a major formulation challenge!

Be aware of the off-flavor potential of protein ingredients
- Degradation during processing
- Selective binding with flavor components (flavor fade)

Consider flavor functionality early in the formulation / development process. Involve your flavor supplier ASAP!

Screen protein ingredients and rebalance flavors as needed to overcome flavor fade to fit your specific application

Utilize masking agents or complementary flavor strategies to overcome inherent off-flavors associated with proteins