Applying Chemistry to Solve Protein Flavoring Issues

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Overview

- A basic understanding of flavor and flavor chemistry
- Impacts of processing on flavor stability
- Sources of protein off-flavors and flavor-protein interactions
- An overview of the chemistry of protein systems and causes of flavor change
- Applications of off-flavor masking agents
Food Products are Complex Systems!

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

- Flavor quality is a major driver of consumer acceptance for food products
- Commercial success of a newly launched food product is directly linked to flavor
- While flavors are present at only trace levels – they exert high impact!
What is Flavor?

The Flavor Experience = Aroma + Taste + Chemesthesis

- **Aroma: Aromatics**
  - Volatile
  - Primarily fat soluble
  - Over 7,000 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 concentrations
- 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
Causes of Flavor Deterioration

- Heating
  - High temperature processing
  - Volatile flash-off
- pH
- Metal ions
  - Iron
  - Copper
- Oxidation of fats – Air / light
- Oxidation of fats – Enzymes
  - Lipoxygenase + fatty acids in soybean oil
- Maillard browning
- Interactions of flavors with food ingredients
  - Cherry flavor with Aspartame
  - Vanilla flavor with whey protein concentrate
Flavors and Proteins

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

1. Imparting undesirable off-flavors
   “Beany” flavors; astringency; chalky 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
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, bitter

• Pea protein
  - Earthy, grassy, nutty, savory; grainy mouthfeel

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

Maillard reactions

<table>
<thead>
<tr>
<th>Compound</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 compounds
- Strecker Degradation: Aldehydes, creatinine

MELANOIDINS
(brown polymeric pigments)
Thermally-Generated Flavors

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

**Flavor Defects**
- Dried / UHT milk
- Whey powder
- Dried sour cream
- Cheese powder
- Soy milk

**Chemical Processes:** Maillard Reactions, Caramelization, Strecker degradation

**COOKING OF FOODS**

**FOOD STORAGE**
(Staling over shelf-life)
Maillard Reaction Parameters

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

Heat Treatment / Shelf-Life Storage

Off-Flavor Formation in Proteins

Maillard reaction chemistry

UHT Milk

Milk powder

Whey powder

Dried sour cream
Marsili, ACS Symp. Ser. 971, 2007

Cheese powder
Marsili, ACS Symp. Ser. 971, 2007

Soy milk
Kwok, Food Sci. Technol., 1995
Thermally Generated Off-Flavors

**SPRAY-DRIED CREAM**

Diacetyl + Arginine \[\rightarrow\] Maillard Rxn. \[\rightarrow\] 2,4,5-Trimethyloxazole ("melon", "ripe kiwi")


**SPRAY-DRIED MILK POWDER**

Tryptophane \[\rightarrow\] Maillard Rxn. \[\rightarrow\] 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 → Maillard Reaction → Strecker Degradation

- 2,6-Dimethylpyrazine ("nutty")
- 2-Ethyl-3-methylpyrazine ("nutty, earthy")
- 2-Ethylpyrazine ("nutty")
- Methional ("boiled potato")

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://chubbylemonscience.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, salt level, degree of denaturation. *Protein denaturation can increase flavor absorption, through greater exposure of hydrophobic regions*

- Result: **Flavor fade** (reduction of flavor intensity)
Flavor-Food Interactions:

Reactions: Flavors + Amino Groups

Benzaldehyde (Cherry) + Aspartame → “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

http://www.chemguide.co.uk/organicprops/aminoacids/proteinstruct.html
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

Hydrophobic pockets:
- Phe
- Leu

Ionic regions:
- NH$_3^+$
- Arg, Gln, Lys
- COO$^-$
- Glu
Binding/Interaction Related to the Type of Protein

Soy > Whey > Gelatin > Casein > Corn
Flavor-Protein Interactions:

Vanilla Binding with Dairy Proteins

Flavor-Protein Interactions:

Vanilla Binding with Dairy Proteins

Source: Chobpattana, W.; Jeon, I. J.; Smith, J. S.; Loughin, T. M. J. 
Flavor-Protein Interactions:

Ketone Binding with Whey Proteins

$\beta$-Lactoglobulin

Flavor-Protein Interactions:

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

Protein - Flavor Applications
Flavor Challenges
High-Protein 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
North America Food & Beverages
2007–2011 Launches

Mintel GNPD
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
## Appropriate Flavor Types

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

- Organic, pasture-fed cows
- Soy milk, whey protein concentrate
- 14-28 g of protein/serving

Flavors: Chocolate bliss, Java Gym Coffee, Chai green tea, Berry Burst
Beyond Meat: Chicken-Free Strips

“Looks, feels, tastes and acts like chicken – without the cluck”
Soy Protein Isolate, Pea Protein Isolate

Flavor system: Chicken flavor (yeast extract), hickory smoke, spices
Flavor Masking

Situations frequently occur where it is necessary to add other flavors to “mask” or cover-up sensory defects.

<table>
<thead>
<tr>
<th>Flavor defect</th>
<th>Food product / ingredient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beany, grassy</td>
<td>Soy beverages, bars</td>
</tr>
<tr>
<td>Harshness, bitterness</td>
<td>Artificial sweeteners</td>
</tr>
<tr>
<td>Astringency</td>
<td>Low pH, whey ingredients</td>
</tr>
<tr>
<td>Vitamin B off-flavor</td>
<td>Vitamin fortification</td>
</tr>
<tr>
<td>Metallic</td>
<td>Mineral 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: “Earthy” 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) and citrus, peach, apple work well with whey protein ingredients; mask whey off-flavor and aroma.

- **Berry flavors** (strawberry, raspberry, blueberry, etc.) are a challenge to use with whey protein ingredients; do not mask whey flavor and aroma as well.

- **Complementary ingredients:** Adding larger carbohydrates such as soluble fiber also may decrease astringency
Consider flavor functionality early in the formulation / development process! Involve your flavor supplier ASAP!

Analytical tools can often measure and diagnose potential causes of flavor-food interactions:
- Degradation during processing
- Cross-reactivity with matrix components

The need continues for practical alternatives to measure flavor interactions with total food system components.

Screen ingredients for their flavor effects using realistic model flavors (appropriate functional types and levels)