Toxicants Formed During Food Processing

Food Toxicology
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Learning Objectives

• Discuss the general principles behind food processing and preparation.
• List the major natural processes modifying food.
• List the major food processing approaches.
• Describe the physical chemistry background of toxicant formation in food processing.
• Describe N-Nitrosamine formation from nitrites.
• Explain the formation of polycyclic aromatic hydrocarbons in cooking.

Food Processing and Preparation: Why

• Preservation allows longer term availability of food.
  – Economic and food availability dimensions: shelf-life.
• Major role in establishing and maintaining microbial food safety (e.g. pasteurization).
• Decreases toxicity of some foods (e.g. lectins beans).
• Conversion into new foods (e.g. cheese, beer).
• Supplementation, fortification of food (e.g. fortified milk).
• Sensory, diversity, nutrition.

Food Processing and Preparation: General

• Addition of thermal energy and elevated temperatures (e.g. cooking, sterilization).
• Removal of thermal energy and reduced temperatures (e.g. frozen foods).
• Removal of water and reduced moisture content (e.g. dried fruit).
• Use of packaging (e.g. canning).
• Mixtures of ingredients (e.g. water).
• Addition of modifiers and additives (e.g. salt, sugar, starch).

Learning Objectives

• Describe amino acid pyrolysates and their formation in cooking.
• Explain the formation of Maillard reaction products.
• Describe Lysinoalanine cross-linkage from alkali/heat treatment of proteins.
• Explore the background and risk assessment of acrylamide formation in foods prepared at high temperatures.

Food Processing and Preparation

• Conversion of raw vegetable, animal, or marine products into food for consumption.
• Preservation of food is the most important reason.
  – Usually by reducing or eliminating microbial contamination.
• Can result in intermediate or final food products.
• Involves labor, energy, machinery, and knowledge.
• Can be commercial or consumer level.

• Description of thermal energy and elevated temperatures (e.g. cooking, sterilization).
• Removal of thermal energy and reduced temperatures (e.g. frozen foods).
• Removal of water and reduced moisture content (e.g. dried fruit).
• Use of packaging (e.g. canning).
• Mixtures of ingredients (e.g. water).
• Addition of modifiers and additives (e.g. salt, sugar, starch).
Natural Processes Modifying Food

- Spoilage and "available" microorganisms (e.g. wine yeasts).
- Atmospheric O₂ oxidation.
- Atmospheric CO₂ pH buffering.
- Food enzyme release (e.g. cassava).
- Post-harvest instability (e.g. potato greening/sprouting).
- Environmental equilibria.
  - Thermal (ambient temperature).
  - Moisture (ambient humidity).
- Contamination.
  - Water, insects, vessels, natural products (green potatoes, weeds).

Food Processing Approaches

- Thermal processing.
- Blanching and pasteurization.
- Sterilization.
- Refrigerated storage.
- Freezing and frozen food storage.
- Liquid concentration.
- Dehydration.
- Physical processes.
  - Mechanical separation.
  - Extrusion.
- Irradiation.

Chemistry of Processing Toxicant Formation

- Chemical thermodynamics and kinetics apply.
- Non-spontaneous reactions can occur at higher temperatures.
- Gibbs free energy change of a chemical reaction.
  \[ \Delta G(J/mol) = \Delta H(J/mol) - T(K) \cdot \Delta S(J/molK) \]
  - Importance of enzymes and catalysts.
- Kinetics of quality change are related to temperature.
  - Arrhenius equation.

Food Processing Toxicants, Pro-Toxicants

- Chemicals added or created during food processing can be anti-nutritive, toxicants, or pro-toxicants.
- Anti-nutritive chemicals or processes will block, interfere, or destroy nutrient availability.
- Toxic chemicals formed from food processing will be dose dependent and subject to biotransformation, sequestration, and elimination.
- Pro-toxicants added or created during food processing can undergo toxication during digestion or biotransformation.

Food Processing and Preparation Toxicants

- N-Nitrosamine formation from nitrites.
- Polycyclic aromatic hydrocarbons.
- Amino acid pyrolysates.
- Maillard reaction products.
- Food irradiation - unique radioytic products (URPs) from ionizing radiation.
- Lipid oxidation products.
- Lysinoalanine cross-linkage from alkali/heat treatment of proteins.
- Acrylamide formation in foods prepared at high temperatures.

N-Nitrosamine Formation from Nitrites

- Nitrite used in curing meat and fish products.
- Has antimicrobial activity, sensory attributes, and reacts with myoglobin and hemoglobin to form red nitrosyl compounds.
- Nitrite reacts with 2º, 3º amines to form stable nitrosamines.
- High temperature processing and protein degradation to 2º, 3º amines increase rate of formation.
- Carcinogenic, mutagenic.
Food Toxicology

Formation of Nitrosamine

\[ \text{NO}_2 + \text{H}^+ \rightarrow \text{HONO} \rightarrow \text{NO}^+ + \text{H}_2\text{O} \]

\[ \text{H}^+ + \text{R}_2\text{NNO} \rightarrow \text{N-Nitrosamine formation} \]

\[ \text{Proline} \rightarrow \text{Nitrosopyrrolidine} \]

Dialkylnitrosamine

\[ \text{Enzymatic } \alpha \text{-hydroxylation} \]

\[ \alpha \text{-Hydroxynitrosamine} \rightarrow \text{Aldehyde} \]

\[ \text{Monoalkylnitrosamine} \rightarrow \text{Diazoalkane} \]

\[ \text{Diazoalkane} \rightarrow \text{Diazohydroxide} \]

\[ \text{RC}_2\text{H}_2\text{N} + \text{Alkyldiazonium} \rightarrow \text{RC}_2\text{H}_2\text{N} + \text{Alkyl} \text{carbonium ion} \]

Polycyclic Aromatic Hydrocarbons (PAH)

- Formed in the high temperature pyrolysis of carbohydrates in grilling and smoking of meats.
- Endogenous food sources and environmental contamination are also important.
  - Products of combustion.
- Carcinogenic, mutagenic.

Polycyclic Aromatic Hydrocarbons

- Benzo[a]pyrene
- Benzo[a]anthracene
- Chrysene
- Benzo[b]fluoranthene
- Bay region 7,8-Epoxide
- 7,8-Diol-epoxide (reactive)
- 7,8-Diol
- DNA

PAH Carcinogenic Activation

Protein Reaction: Processing and Storage

- Oxidizing Lipids
- Reducing Sugars
- Phenols
- Nutritional Changes
- Possible Toxicity
- Lysine
- Methionine
- Cystine
- Tryptophan
**Simplified Scheme of the Maillard Reactions**

- **Reduction** + Amino Compound
- **Dehydration**
- **Fission**
- **MELANOIDIN FORMATION** by the polymerization of intermediate compounds, production of N-heterocyclics

**Maillard Reaction: Non-Enzymatic Browning**

- **Coffee**
- **Bread**
- **Cocoa**
- **Cooked meats**
- **Beer**
  - Desirable color, flavor and aroma (pyrazines, aldehydes)
- **Milk**
  - Undesirable color, flavor

**Amino Acid Pyrolysates**

- Heterocyclic aromatic amines (HCAs) formed during broiling of meat, fish, or other high protein-rich foods.
- High temperature thermal degradation products of tryptophan (β-carbolines) and other amino acids (imidazo-quinoline or imidazo-quinoxalin-2-amine derivatives - IQ compounds).
- Also formed from the reaction of Maillard products (pyridines or pyrazines, and aldehydes) with creatinine.
- Mutagenic (form DNA adducts).

**Formation of β-Carbolines**

- Free Tryptophan
- β-Carboline
- Schiff-base

**Imidazo-Quinolines & Imidazo-Quinoxolines**

- 2-amino-3,8-dimethylimidazo (4,5-f)quinoline
- 2-amino-3-methylimidazo (4,5-f)quinoline

**Imidazo-Quinolines & Imidazo-Quinoxolines**

- 2-amino-3,8-dimethylimidazo (4,5-f)quinoline
Meat Mutagens

- Over 20 meat HCAs have been shown to cause cancer in laboratory animals when administered at high doses.
- Form DNA and protein adducts.

Lysinoalanine in Food

- Cross-linked lysine arising from alkali and heat treatment of proteins.
- Little influence on available lysine.
- Reduced protein digestibility.
- Strong affinity for copper and other metal ions (enzyme inactivation).
- Main concern is toxicological.
- Renal cytomegaly in rats.

Alkali Treatment of Food Proteins

- Used for extraction, functional properties (solubility)
- Mild treatment (<pH 9) - no damage
- >pH 10 - damage

Nutritional and Toxicological Concerns

- Lysine
- Cysteine
- Threonine

Almost complete in strong alkali, Only L-amino acid utilized

Renal lesion in rats
- CYTOMEGALY (enlarged nucleus)
- Increased DNA

Lysinoalanine: Alkali-Treated Proteins

Cysteine

Dehydroalanine

Lysinoalanine Cross-Linkage

Lysinoalanine in Food

<table>
<thead>
<tr>
<th>Commercial Food Ingredients (Alkali Treated)</th>
<th>LAL mg/kg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy protein isolate</td>
<td>0-370</td>
</tr>
<tr>
<td>Sodium caseinate</td>
<td>430-6,000</td>
</tr>
<tr>
<td>Whipping agent</td>
<td>6,000-60,000</td>
</tr>
</tbody>
</table>

Home-cooked foods

- Chicken: 0-170
- Egg white: 140

Commercial foods

- Corn chips: 390
- Pretzels: 500
- Milk powder: 0

Liquid infant formula

- UHT: 160-370
- In can sterilized: 410-1,030
**Acrylamide in Food**

- 2000-2002 Swedish researchers identify acrylamide (ACR) in foods and residues from human samples.
- Acrylamide is a neurotoxin and carcinogen.

\[
\text{HC}=\text{NH}_2
\]

Acrylamide

**Acrylamide Uses**

- Cement binder
- Plastic manufacture
- Waste water treatment (flocculent)
- Soil conditioner (prevents erosion)
- Thickening agent for pesticides
- Refining sugar (flocculent)
- Cosmetics
- Ore processing
- Laboratory gels (PAGE)
- Polyacrylamide in food packaging

**Toxicology: Pre-Food**

- Known neurotoxicant.
  - Peripheral neuropathy.
  - Tingling/numbness of extremities.
  - Loss of reflexes.
  - Chronic = CNS dysfunction and neuropathy.
- Reproductive toxicity.
- Animal carcinogen (CNS, endocrine organs)
  - Mice 10X more than rats.
- Probable human carcinogen
  - Interagency for Cancer Research (IARC, 1994).
- Biomarker – adducts on valine aa of Hb.

**Mechanism of Action - Carcinogen**

- Epoxide formation via P450s.
- Glycidamide metabolite.
- Binds to SH groups on critical enzymes and amino acids and DNA.
- Detoxified via glutathione-s-transferase, Phase II.
- Conditions of protein deficiency exacerbate, due to low GSH.
  - Malnutrition, oxidative stress and liver damage.

**Mechanism of Action - Neurotoxicant**

- Disruption of kinesin proteins involved in signal transduction - nerve cells die back – may be related to repro tox and cancer.
- Interference with membrane fusion process at nerve terminus – synaptic vesicles cannot fuse to conduct signals cannot be conducted nerve dies.

**History - Food Related**

- Tunnel workers in Sweden – waterproof sealant with ACR-developed neurotoxicity.
- Observed acrylamide-Hb adducts in controls.
- Hypothesized a food source, maybe fried due to formation in burning tobacco.
- Rat feeding study of fried and non-fried foods.
  - Fried food group had higher Hb adduct levels.
  - Tareke et al. 2000
- Led to more detailed studies of food levels.
### History
- Broad range of commercial foods with significant levels of acrylamide.
  - Foods prepared at high temperatures.
  - Fried and baked but not boiled.
  - Higher in high carbohydrate foods.

### International Activity
- FAO/WHO Expert Consultation/Seminar
  - Geneva, Switzerland, June 2002
  - Tanzania, March 2003
- Acrylamide in Food Workshop: JIFSAN
  - Chicago, Oct. 2002
- FDA Public Meeting/Advisory Committee
  - Sept. and Dec. 2002; Feb. 2003
- EU Meetings/Workshop
  - July and October 2002; March 2003
- Additional meetings.

### Acrylamide Levels in Foods (μg/kg)

<table>
<thead>
<tr>
<th>Food</th>
<th>Levels (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread Products</td>
<td>&lt;10-130</td>
</tr>
<tr>
<td>Bread Products (toasted)</td>
<td>246-384</td>
</tr>
<tr>
<td>Crackers/Biscuits</td>
<td>26-620</td>
</tr>
<tr>
<td>Cookies</td>
<td>36-432</td>
</tr>
<tr>
<td>Breakfast Cereals</td>
<td>11-1067</td>
</tr>
<tr>
<td>French Fries</td>
<td>117-1325</td>
</tr>
<tr>
<td>Potato Chips</td>
<td>117-2752</td>
</tr>
<tr>
<td>Tortilla Chips</td>
<td>11-220</td>
</tr>
<tr>
<td>Popcorn</td>
<td>157-181</td>
</tr>
<tr>
<td>Coffee (ground)</td>
<td>37-374</td>
</tr>
<tr>
<td>Coffee (brewed)</td>
<td>5-11</td>
</tr>
<tr>
<td>Cocoa</td>
<td>ND-909</td>
</tr>
<tr>
<td>Nuts</td>
<td>ND-457</td>
</tr>
<tr>
<td>Peanut Butter</td>
<td>64-125</td>
</tr>
<tr>
<td>Frozen Vegetables</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Canned Fruits/Vegetables</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Mashed Potatoes</td>
<td>ND</td>
</tr>
<tr>
<td>Infant Formula</td>
<td>ND</td>
</tr>
</tbody>
</table>

### Mechanism of Acrylamide Formation
- Acrylamide derived from asparagine (amino acid) in the presence of sugar.
- Carbonyl or C=O (glucose) facilitates reaction (Maillard-type).
- High asparagine, sugar and temp = high acrylamide.

### Acrylamide to Glycidamide in vivo
- Actual carcinogen is the epoxide, glycidamide.

### Time - Temperature Relationship
Changes in acrylamide levels in French-fries with increasing temperature and frying time.

[Diagram showing changes in acrylamide levels with temperature and frying time.]
Estimated Exposure from Food

- Calculated acrylamide intake.
  - FAO/WHO: 0.3 - 0.8 μg/kg body weight/day.
  - FDA: 0.37 μg/kg body weight per day (mean).
  - Common average used is 1 μg/kg bw/da.
- No one food accounts for the majority of the mean population intake.
  - Foods with lower levels/high consumptions contribute significantly to estimated intake.

Acrylamide - Risk

- Levels consumed are 1000X lower than levels causing neurotoxicity in humans.
- Reference dose = 12 μg/kg bw/day.
- 10X safety factor from reproductive studies in rats.
- No adverse epidemiologic evidence for problem.

Epidemiologic Studies: Pre-Food

- Marsh et al. 1999: same as Collins but 11 yrs later.
- No associations with any kind of cancer.

Epidemiologic Studies: Post-Food

- Mucci 2003: 1500 Swedes, bladder, kidney, colon cancer, 14 different foods.
- Mucci 2004: 60,000 women, colon and rectal cancer.
- Mucci 2005: 49,000 women, breast cancer.
- Daily intake est. 40 μg/day.
- *No relationship to any cancers.
- Pelucchi et al 2003: no relationship with cancer and fried potatoes, 10 yr.
- Two studies found decrease in colon cancer.
- More studies in progress.

Methods to Minimize in Food

- Do not over-cook high carbohydrate foods.
- Avoid foods high in asparagine and sugar.
- Decrease asparagine levels in foods via genetic manipulation.
- Hydrolyze asparagine with acid or amidases.
- Acetylate asparagine to prevent formation of glycoside intermediates with sugar.
- Research conditions that limit acrylamide formation.

General Recommendations

- Insufficient evidence to warrant significant change to the existing dietary recommendations...
- FDA...continued emphasis on “a balanced diet, choosing a variety of foods that are low in trans fat and saturated fat, and rich in high-fiber grains, fruits, and vegetables.”
- FAO/WHO...“reinforces general advice on healthy eating”...
  - Advises “foods should not be cooked excessively...for too long or at too high a temperature... However, all food...should be cooked thoroughly to destroy foodborne pathogens.”
Some bacteria can synthesize or degrade acrylamide.
– May be involved in decreased or increased exposure.

Highest levels from plant foods.
– Hardly any from animal.

Levels vary between same foods based on cooking temperature and time, frying oil, nature of food matrix, etc.

Several other aa can contribute to ACR levels but very minor.