Toxicants Formed During Food Processing
Food Toxicology
Instructor: Gregory Möller, Ph.D.
University of Idaho

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.

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.
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).

Natural Processes Modifying Food

• Spoilage and “available” microorganisms (e.g. wine yeasts).
• Atmospheric O$_2$ oxidation.
• Atmospheric CO$_2$ 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 radiolytic 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 nitrosoamines.
• High temperature processing and protein degradation to 2º, 3º amines increase rate of formation.
• Carcinogenic, mutagenic.

Formation of Nitrosamine
Nitrosamine: Alkylating Agent Formation
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
PAH Carcinogenic Activation
Protein Reaction: Processing and Storage
Simplified Scheme of the Maillard Reactions
Maillard Reaction: Non-Enzymatic Browning
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**
**Imidazo-Quinolines & Imidazo-Quinaxolines**

**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.
• 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

**Lysinoalanine: Alkali-Treated Proteins**

**Lysinoalanine: Non-Alkali Treated**

**Lysinoalanine in Food**

**Acrylamide in Food**

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

**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 neurtoxicant.
  – 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 reproductive toxicity and cancer.
• Interference with membrane fusion process at nerve terminus – synaptic vesicles cannot fuse – 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
• 2002 Swedish press release.
• 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
• EU Meetings/Workshop
  – July and October 2002; March 2003
• Additional meetings.

Acrylamide Levels in Foods (μg/kg)

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
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 1µg/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
• Sobel et al. 1986: 371 workers in ACR plants.
• Collins et al. 1989: 8500 workers in ACR plant.
• 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.”

Acrylamide - Other
• 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.