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.

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.

Heldman & Hartel

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).

7

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.

8

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.

9

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.

10

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.

11

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.

12

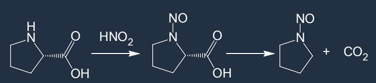
Formation of Nitrosamine

$NO_2 + H^+ \rightleftharpoons HONO \rightleftharpoons NO^+ + H_2O$

$\downarrow R_2NH$

$H^+ + R_2NNO$

N-Nitrosamine formation

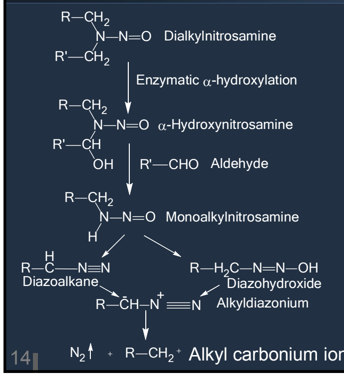


Proline Nitrosopyrrolidine

Dimethylnitrosamine Diethylnitrosamine

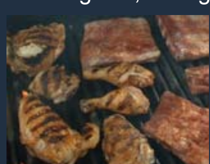
Nitrosamine: Alkylating Agent Formation

Shibamoto
Bjeldanes

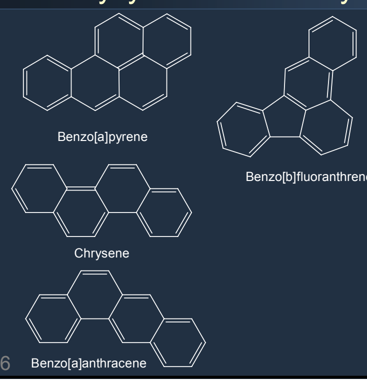


Polycyclic Aromatic Hydrocarbons (PAH)

- 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.

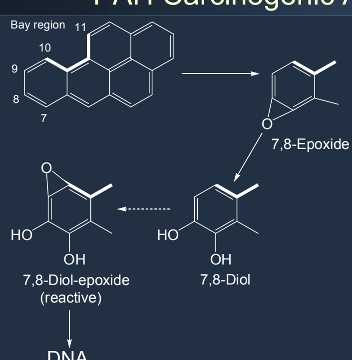


Polycyclic Aromatic Hydrocarbons



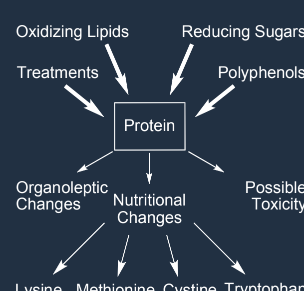
PAH Carcinogenic Activation

Marquardt

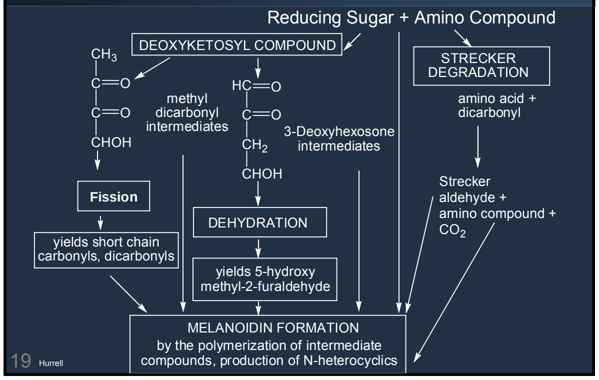


Protein Reaction: Processing and Storage

Hurrell



Simplified Scheme of the Maillard Reactions



Maillard Reaction: Non-Enzymatic Browning

Coffee
Bread
Cocoa
Cooked meats
Beer

Desirable color, flavor and aroma (pyrazines, aldehydes)

Milk
Infant food

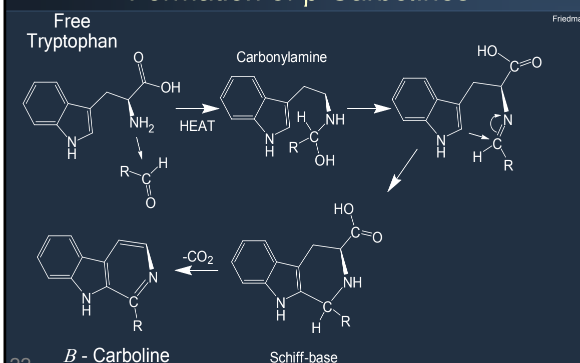
Nutritional losses
Undesirable color, flavor

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).

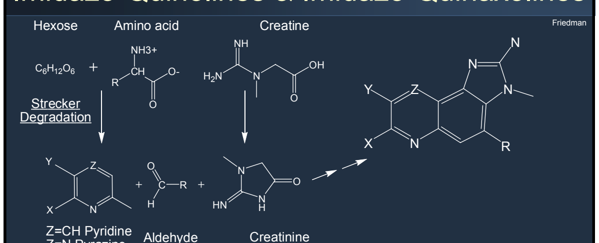
Formation of β-Carbolines

Friedman



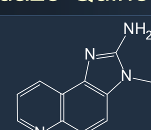
Imidazo-Quinolines & Imidazo-Quinaxolines

Friedman



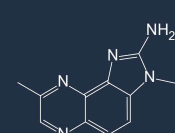
	X	Y	Z	R
IQ	H	H	CH	H
MeIQ	H	H	CH	Me
MeIQx	H	Me	N	H
7, 8 diMeIQx	Me	Me	N	H
4, 8 diMeIQx	H	Me	N	Me

Imidazo-Quinolines & Imidazo-Quinaxolines



IQ

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

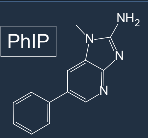


MeIQx

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

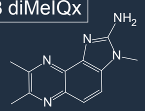
Meat Mutagens

PhIP



2-amino-1-methyl-6-phenyl imidazo (4,5-b) pyridine

7,8 diMeIQx

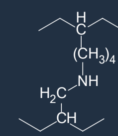


2-amino-3,7,8-trimethyl imidazo-(4,5-f) quinoxaline

- 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

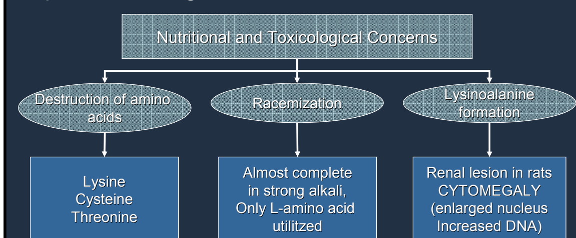
- Cross-linked lysine arising from alkali and heat treatment of proteins.
- Little influence on available lysine.
- Reduced protein digestibility.
- Strong affinity for copper and other metal ions (enzyme inactivation).
- Main concern is toxicological.
- Renal cytomegaly in rats.



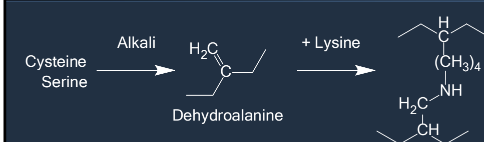
Lysinoalanine

Alkali Treatment of Food Proteins

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

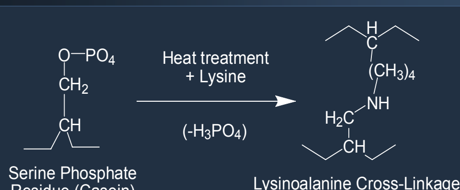


Lysinoalanine: Alkali-Treated Proteins



Lysinoalanine Cross-Linkage

Lysinoalanine: Non-Alkali Treated

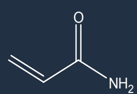


Lysinoalanine in Food

Commercial Food Ingredients (Alkali Treated)	LAL mg/kg protein
Soy protein isolate	0-370
Sodium caseinate	430-6,900
Whipping agent	6,500-50,000
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.



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

Exon

- 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.

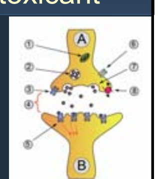
Mechanism of Action - Carcinogen

Exon

- 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 ② cannot fuse ⑦ signals cannot be conducted ④ nerve dies.



Nerve Cell Bundle

History - Food Related

Exon

- 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.
    - J. Agric. Food Chem. 50:4998 (2002)

International Activity

Exon

- 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.

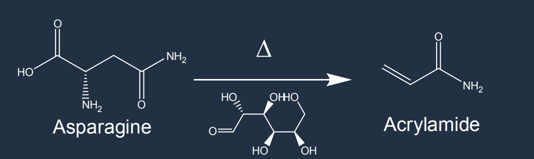
Acrylamide Levels in Foods (μg/kg)

FDA

Bread Products	<10-130
Bread Products (toasted)	216-364
Crackers/Biscuits	26-620
Cookies	36-432
Breakfast Cereals	11-1057
French Fries	117-1325
Potato Chips	117-2762
Tortilla Chips	11-220
Popcorn	157-181
Coffee (ground)	37-374
Coffee (brewed)	5-11
Cocoa	ND-909
Nuts	ND-457
Peanut Butter	64-125
Frozen Vegetables	<10
Canned Fruits/Vegetables	<10
Mashed Potatoes	ND
Infant Formula	ND

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.



Asparagine Glucose Acrylamide

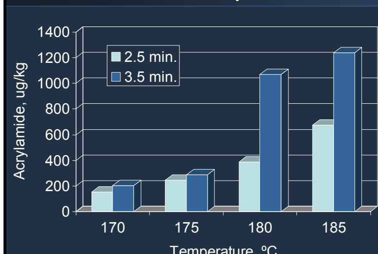
Acrylamide to Glycidamide *in vivo*

Metabolism

Acrylamide Glycidamide

- Actual carcinogen is the epoxide, glycidamide.

Time - Temperature Relationship



Changes in Acrylamide levels in French-fries with increasing temperature and frying time

Gertz and Klosterman (2002) Eur. J. Lipid Sci. Technol. 104:762-771.

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.

Exon

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.

Exon

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.

Exon

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.

Exon

Methods to Minimize in Food

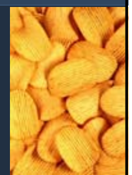
- Do not over-cook high carbohydrate foods.
- Avoid foods high in asparagine and sugar.
- Decrease asparagine levels in foods via genetic manipulation.
- Hydrolyze asparagine with acid or amidases.
- Acetylate asparagine to prevent formation of glycoside intermediates with sugar.
- Research conditions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

Exon

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.