Working Group #1 Mechanisms of Formation and Methods of Mitigation



2nd Acrylamide in Food Workshop Chicago · April 13-15, 2004

- **B. Food categories**
- C. Methods of Mitigation Control / Intervention Points



WG1 – Formation & Mitigation 2^{nd} Acrylamide in Food Workshop Chicago \cdot April 13-15, 2004

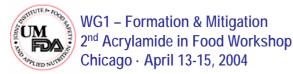
1. Carbonyls + Asparagine \rightarrow acrylamide (AA)

Three potential pathways from the Schiff base

- 1.1 Oxazolidin-5-one intermediate, decarboxylation, rearrangement to the Amadori and beta elimination to AA.
 - Pathway marginal versus Amadori (1-5%).
 - Rate limiting step beta elimination. General applicability of the beta elimination pathway to other amino acids shown (phenylalanine to styrene, asp to acrylic acid, other vinylogous compounds).
 - So far no evidence for the oxazolidin-5-one nor azomethine ylide intermediate.
 - Impact of pH ?
- 1.2 Decarboxylation and 1 cleavage of the imine to AA
 - Or via 3-APA.
 - Rate limiting step is decarboxylation reaction. Gluc \rightarrow 3-APA



- 1. Carbonyls + Asparagine \rightarrow acrylamide (AA)
 - 1.3 3APA so far identified and quantified in potatoes
 - Efficient yield of AA from 3APA (60%) in high moisture models (Prof. Schieberle, Munich).
 - Direct decarboxylation from free Asn also possible
 - Potential of this intermediate to be involved in formation of AA under pressure cooking conditions ?



- 2. Aspartate + Sugar \rightarrow acrylic acid + NH₃ \rightarrow acrylamide
 - Minor pathway, e.g., asparaginase conversion of Asn →Asp led to 97% ↓ AA in potato crisps
 - Aspartic acid $\rightarrow \beta$ -alanine \rightarrow acrylic acid
 - (C1 decarboxylation favored)
 - β -alanine is more efficient than acrylic acid + NH₃ in generating AA
- 3. Triglycerides $\rightarrow \rightarrow \rightarrow$ acrolein \rightarrow acrylamide
 - Potential source of carbonyls (see Mechanism 1.)
 - Certain "cold-pressed" oils can lead to AA upon heating (wheat germ)



4. Other

- Meat systems: Carnosine \rightarrow beta alanine \rightarrow acrylic acid
- In the presence of creatine lead to N-methylacrylamide and N,Ndimethylacrylamide
- Acrylic acid + methylamine \rightarrow N-methylacrylamide
- Serine and cysteine → acrylic acid (via pyruvic acid → lactic acid → acrylic acid)
- 5. Prevalence of each: *Pathway 1 is the Major Route*.



B. Food categories

- 1. Contains acrylamide
- 2. Significant in diet
- 3. "Top Eight"

French fries (FF), French fries (OB), breakfast cereals, potato chips, cookies, coffee, toasted bread, pies and cakes

Additional focus on infant foods - baby foods in jars, teething biscuits

Recognize uniquely high products, encourage research - gingerbread, coffee substitutes, etc.



Potato crisps

- Potato \rightarrow potato flakes \rightarrow potato crisp
- Moisture control: increase 0.5% gives mean 20% reduction
- Sugar control: \checkmark 15% = \checkmark 15% AA
- Acidity control: \checkmark pH = \checkmark AA <u>and</u> \checkmark consumer acceptance
- Blanching: ↓ AA ≈80%
- Asparaginase (A. oryzae): ↓AA 97% in lab; color, flavor acceptable
 - Collaboration with Novozyme on enzyme standardization
- Challenges of scale up: AA reduction "lost" in variability (RSD 15%, 2-σ 28 %)
- Several years to commercialization



<u>Almonds</u>

- Lab test results are preliminary:
 - Temperature more critical than time
 - AA formation uses basically all sugars, only a portion of free Asn
 - AA formation correlates with free Asn, which varies greatly
 - AA may correlate with color (Hunter L)
- Process mitigation is complex due to multiple applications and customer requirements



Breakfast Cereals

- Test:
 - Product: rotary cooked, flaked wheat breakfast cereal
 - Intervention: process steps, not raw materials
- Earlier work showed AA formed mainly during the toasting step
- Pre-drying flakes:
 - Reduced AA but relationship non-linear
 - More fragile product broke more easily, higher manufacturing losses.
 - Negative impact on flavor
- No practical means to control flake moisture
- Great variability within and between lots
- No practical strategies that reduce AA and maintain key sensory attributes





Potato slices

- Objective: Prevention of formation, manipulation of the reaction
- Physical removal (steam distillation), chemical reaction
- Effects of pretreatments
 - Surface washing = \checkmark 28% AA
 - Basil/rosemary/thyme = $\sqrt{35\%}$ AA
 - Blanching = no impact
- Competing amino acids, additive effects Glu/Asp = $\sqrt{43\%}$ AA
- Problem of scale up
- Future work: apply recent information on effects of Ca⁺⁺ and phytate?



Effects of Consumer food preparation

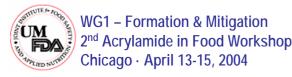
- % AA exposure due to home preparation unknown
- AA increase with temp., frying time, color (browning)
- Toasted bread : AA increase with degree of darkness. 2/3 reduction by scraping off the surface !
- Home storage of potatoes: less AA at room temperature vs. cold
- French fries: soaking in water 15 min. (2/3 reduction)
- Consumer acceptability still to be studied
- Precision of home fryers, ovens a confounding variable in application of any consumer message.



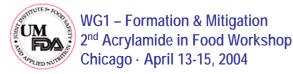
- 1. What have we accomplished so far?
 - Importance of free Asn, carbonyls from other sources
 - Major route identified, with three possible sub-routes
 - Role of other amino acids, e.g., carnosine in meat
 - Importance of ammonium compounds in baked wares
 - Good progress and data on potato chips and French fries
 - AA decline in stored coffee potential mitigation mechanisms ?
 - Malted beverages, dark beers: no problem (low ppb levels)



- 2. What gaps are remaining?
 - Identification of the key intermediates
 - Quantitation of precursors in foods
 - Kinetics, rate limiting steps = identification of control points
 - Yeast *vs.* chemical leavening in baked goods
 - Understand AA formation in closed, high moisture systems, e.g., retorted baby foods, prune juice, black olives
 - Raw commodity patterns: variation within and between cultivars, seasonal variation
 - Formation of AA adducts with amino acids, proteins in foods, stability in storage, digestion



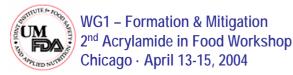
- 3. What are the uncertainties?
 - Consequences of "slowing down" Maillard reaction, lower quantities of "beneficial" compounds?
 - Consequences of mitigation measures, potential creation of other problem compounds *Question to WG Risk Characterization*
 - How specific is asparaginase ?



- 4. Are we covering all the territory? If not, is it covered by another Working Group? If so, is anything being duplicated in another Working group?
 - EU HEATOX project will study formation mechanisms and kinetics of AA in different foods (potato, cereal/bread, coffee)
 - Recommend close communication/interaction with HEATOX team:
 - Feasibility, impact of proposed mitigation measures
 - Impact of mitigation on the formation of other "undesirable" compounds.
 - Will any JIFSAN Workshop measures cause concern for HEATOX?
 - Effective communication of formation/mitigation learnings to:
 - Smaller producers, private label producers, co-packers
 - Restaurant/food service and retailers with in-store preparation



- 5. What research is needed?
 - See question 2. What gaps are remaining? above
 - Advanced glycation end products
 - Role of water
 - Steam distillation of AA away from foods during processing
 - "Closed" processing situations, e.g., retorted or pasteurized products
 - What mechanisms drive AA decline in stored roasted coffee? Guide for other products, processes?



WG1-Specific Questions

- 1. What is a realistic reduction?
 - Modest reductions have been achieved in Europe in certain food categories, potato products, crisp bread, baby biscuits. In others, considerable hurdles (breakfast cereals, coffee). Impact of seasonality not adequately studied
 - Process controls have had minor effects, no substantial further reduction without compromising quality or fundamental changes in raw materials, pretreatment or processing.



WG1-Specific Questions

- 2. Does this reduction significantly reduce the exposure to acrylamide from food?
 - What reduction is necessary to reduce the risk ? (Question to WG Toxicology)
 - Need concerted reduction for all foods, removal from one food product category will not impact overall average consumption (population)
 - Reduction in selected products could lower intake for certain individuals



WG1-Specific Questions

- 3. What is a realistic date(s) to achieve the target?
 - Several years to commercialize asparaginase treatment for selected potato products
 - Expect progress in "small steps"
- 4. Can we predict based on the knowledge of the mechanisms which other foods could be problematic ?
 - Apply established kinetic models (e.g. Bronek Wedzicha)
 - Measurement of intermediates (retorting, formation in certain foods under high moisture conditions)

