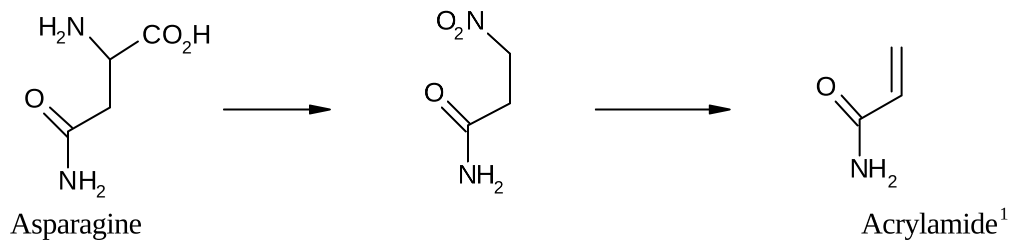

Working Group 1:
Mechanisms of Formation of Acrylamide in Food

Summary Report

[1] Proposed Mechanism of Acrylamide formation from asparagines based on mass spectral data. Dr Bryan Hanley, Leatherhead Food International, Randalls Road, Leatherhead, Surrey, KT22 7RY, UK.

Format of Mechanisms Working Group Meeting

Objective of the Working Group -- The objectives were (a) to compile, discuss, and reconcile existing data on acrylamide formation in foods, and (b) to provide the sharpest available snapshot of knowledge related to acrylamide issues in food including: identifying likely pathways of formation, rejecting theorized but unlikely pathways, identifying possible mitigation strategies, and identifying immediate research needs. The mechanisms group nominated the four most urgent research needs to a general list of goals compiled by all five Working Groups.

Discussion Format –Discussions were recorded by a scientist-rapporteur and compiled with group collaboration into a reporting document on the final day of discussion.

Presentations – Scientists who published acrylamide mechanisms in recent peer-reviewed literature reported on their work and proposed mechanisms for acrylamide formation. The presentations of Drs. Mendel Friedman, Don Mottram, Richard Stadler and Bryan Hanley are included..

Summary

The presentation by Mottram (Mottram and Wedzicha) and Stadler provide excellent summaries of acrylamide formation pathways from asparagine. The pathway suggests that acrylamide arises from reaction of asparagine with an Amadori dicarbonyl through a Strecker aldehyde intermediate. Stadler provided clear evidence of acrylamide formation from pyrolytic products of N-glucosides of asparagine and, to a much lesser extent, with methionine and glutamine.

Becalski showed in a sealed model system at 175°C that monocarbonyls (octanal and 2-octanone) also produced measurable acrylamide from asparagine hydrate. Acrylamide yields were 11% and 0.7% of the yield for glucose/asparagine hydrate under the same treatment conditions. Zyzak independently confirmed the ability of monocarbonyls to mediate acrylamide formation. Since monocarbonyls are pyrolytic and/or oxidative decomposition products of fats, proteins and carbohydrate, their varied origin suggests that a variety of minor pathways may feed acrylamide formation.

Panel members felt generally confident that free asparagine and carbohydrates (especially free reducing sugars) accounted for the majority of acrylamide in fried potato products. Mottram presented data which indicated a high level of free asparagine content in potatoes (40% of total free amino acids), wheat (14% of TFAA) and high protein rye (18% of TFAA); suggesting a connection between free asparagine and acrylamide formation in foods with moderate-to-high reported values for acrylamide.

The formation of acrylamide from amino acids other than asparagine was not clear-cut. Some acrylamide has been reported from glutamine, methionine, cysteine and aspartic acid. However, Becalski reported finding 0.3% asparagine impurity in aspartic acid and 0.8% cysteine in glutamine. Impurities in stock amino acids almost certainly account for some reported acrylamide. The production of acrylamide from methionine appears genuine. While this mechanism probably represents a minor pathway in foods of plant origin, its significance in forming acrylamides in meats is yet to be tested.

The group agreed that fats in general and acrolein in particular were not major precursors to acrylamide. However, oxidized fats could compete with other carbonyl compounds for a trivial role in acrylamide formation through the asparagine/carbonyl pathway discussed above.

Most published acrylamide studies are the product of research using model systems of pure amino acids and not actual foods. However, attendees reported several experiments performed in real food systems. Asparaginase (converts asparagine to aspartic acid) reduced acrylamide to low levels in fried pureed potato foods. Disruption of cell membranes was necessary to liberate asparagine and allow it to contact asparaginase. Other work examined the effectiveness of reducing agents such as sodium bisulfite, cysteine and glutathione on acrylamide reduction in bakery products. Reducing agents did not appear to significantly effect acrylamide production. At best, levels were reduced by no more than 10%. Similarly, antioxidant supplementation in wheat dough did not significantly decrease acrylamide formation in bakery products.

There was an early temptation among group members to think that virtually all food-borne acrylamide originated with free asparagine perhaps with minor contributions by other free amino acids. This may yet prove to be the case. However, participants acknowledged that little work had examined whole proteins, or other nitrogen sources such as nucleic acids and glycoproteins, and virtually no mechanism work has been published on acrylamide mechanisms in whole foods. One opinion expressed the possibility that free radicals may participate There was a strong feeling that kinetic data would be essential in developing reduction strategies for acrylamide.

The list of four urgent needs for acrylamide mechanisms research were:

1. Need data on the quantity of free asparagine on a dried weight basis for various foods (database-variety, crop conditions, storage) and data on the quantity of glucose, fructose (and other sugars) and amino acids other than asparagine for various foods. (**comment: It was felt that much of this information existed in the literature and might of itself answer whether asparagine is the only significant pathway to acrylamide production in foods)**

2. Time/Temperature/pH/Moisture/Surface area-mass mapping and kinetics of asparagine/carbonyls reaction in various matrices. May include mathematical modelling. Process investigations and study the kinetics/pathways of acrylamide formation versus the browning and flavor forming reactions. (**comment: This**

information addresses the question of formation kinetics and spatial placement of acrylamide in fried and baked foods. Some mitigation strategies depend of whether acrylamide forms on the surface, immediately below the surface or progresses as cooking advances at some mobile interfacial point of optimal temperature and moisture).

3. Define the direct correlation of asparagine to acrylamide production in foods. **(Comment: Experimentally confirms literature effort reported in objective 1 by analysing foods for free asparagine and examining correlation with acrylamide formation)**

4. What are the kinetics of acrylamide inhibition/destruction/scavenging under various reaction/process conditions.

    a. Mixed amino acids competitive reactions or scavenging.
    b. Ammonium ion as a possible competitive agent.
    c. Glutathione/cysteine to promote sulfhydryls-disulfide interchange to provide scavengers.
    d. Irradiation
    e. Pressure processing
    f. Fermentation (e.g. yeast).
    g. Hydrolyzed nucleic acids
    h. Asparaginase conversion of asparagine to aspartic acid.

    (Comment: examines the effectiveness acrylamide mitigation strategies in foods.)

Full List of Proposed Research (not ranked by priority)

1. Need data on the quantity of free asparagine on a dried weight basis for various foods (database-variety, crop conditions, storage).
2. Need data on the quantity of glucose, fructose (and other sugars) and amino acids other than asparagine for various foods.
3. Define the direct correlation of asparagine to acrylamide production in foods.
4. Investigate the effects of production of acrylamide in meats from methionine (and cystine).
5. Time/Temperature/pH/Moisture/Surface area-mass mapping and kinetics of asparagine/carbonyls reaction in various matrices. May include mathematical modeling.
6. Does irradiation of foods containing free asparagine and carbonyls result in the formation of acrylamide.
7. Pressure processing effects on the formation of acrylamide.
8. Investigate the purity of amino acids (e.g. glutamine or aspartic acid) used in model systems for studying the mechanism of formation of acrylamide.
9. What is the effect of fermentation on the quantities of asparagine in foods and subsequently on acrylamide production?
10. Do acrylamide formation intermediates, (e.g. the Schiff's bases), form during storage of potatoes as the asparagine and glucose levels go up?
11. Other mechanisms of acrylamide formation, i.e. directly from protein.

Recommendations for Acrylamide Mitigation Research

1. For potatoes (and perhaps other foods) storage conditions should be investigated for ultimate effect on acrylamide formation.
2. What are the kinetics of acrylamide destruction under various conditions.
3. Investigate mixed amino acids, i.e. do competitive reactions and/or scavenging reactions reduce the free acrylamide. Is ammonium ion (i.e. ammonium carbonate or ammonium bicarbonate) a possible competitive agent? Glutathione/Cystine to promote sulfhydryls-disulfide interchange to provide scavengers?
4. Reduction of free amino acids in matrices by yeast or other organisms and mechanisms
5. Hydrolyzed nucleic acids as a scavenging agents for acrylamide
6. Asparaginase conversion of free asparagine to aspartic acid? Can asparagine be converted/transformed to other compounds of lesser concern, i.e. esters or other derivatives?
7. Modification of biosynthesis pathways to control the formation of free asparagine and/or glucose. (Horticulture, breeding, genetic modification). Is asparagine essential for the survival of the plant.
8. Modification of biosynthesis pathways to control the formation of cystine and/or glutathione as trapping agents. (Horticulture, breeding, genetic modification).

9. Process investigations of Time/Temperature/pH/Moisture/Surface area-mass of asparagine/carbonyls reaction in various matrices. Study the kinetics/pathways of acrylamide formation versus the browning and flavor forming reactions.

Related Acrylamide Questions

1. Is acrylamide forming *in vivo*? What is its fate?
2. What are the nutrition impacts of acrylamide related reactions in foods, i.e. mitigation by elimination of precursors, or reaction of acrylamide in the food matrix? Asparagine is currently considered nonessential amino acid, so it is anticipated that elimination of asparagine will not significantly impact nutrition.
3. Impact of asparagine on food flavors?
4. Investigation of *in vivo* versus *in vitro* reactions of acrylamide (i.e. reactions with proteins and nucleic acids).

At the Close of the Workshop, Working Group Participants Made the Following Summary Statements.

1. Time, temperature, pH and moisture content during processing impact the quantity of acrylamide formed during the cooking of foods. Additional research is necessary to fully delineate this impact on various food matrices.
2. The production of acrylamide via the acrolein and the acrylic acid pathways has been essentially discounted as mechanistic pathways to significant acrylamide formation by various researchers.
3. Fat is not a major player in acrylamide formation *per se*, but merely serves as a thermal transfer medium.
4. The reaction of asparagine with reducing sugars appears to be the predominant mechanism via the Maillard reaction leading to the formation of acrylamide during heating.
5. The reaction of asparagine to lead to acrylamide has further been shown to take place with any readily available carbonyl including non reducing sugars (i.e sucrose), perhaps by a reducing sugars intermediate.
6. Methionine is believed to be the second most significant precursor amino acid to acrylamide formation. Glutamine and cystine have also been shown to produce acrylamide in vitro with glucose, but the purity of the amino acids must be investigated.
7. In plant products the asparagine mechanism is believed to be the major contributor to the final acrylamide content with methionine being a minor contributor. In foods of animal origin, other mechanisms may be important and need to be investigated.
8. Additional study of is needed to fully define the mechanism on a broad range of foods.
9. Preliminary experimentation with asparaginase has been shown to convert the free asparagine into aspartic acid, with a subsequent decrease in acrylamide formation in a model potato system.
10. Preliminary studies with cysteine has been shown to reduce the quantity of acrylamide as has lysine (but not unequivocally). Possible reaction mechanism is reaction with the acrylamide and/or the precursor carbonyl compounds.
11. Preliminary data indicate the acrylamide levels in some matrices do not decrease over time in storage. Additional research is recommended.
12. The effect of light and ionizing radiation on acrylamide in foods is not known. (Formation and/or destruction?).
13. Preliminary data on the effects of select additives on acrylamide formation have not shown positive results. Additions of bisulfite and other antioxidants have not resulted in decreased levels of acrylamide being formed.
14. Each food or food matrix will require individualized research attention with regard to acrylamide formation.