

Report WG1

Methods of Formation and methods of mitigation

A. Mechanisms of formation

Significant progress has been made in the past year in understanding how acrylamide (AA) is formed in foods. There is today consensus that the presence of free asparagine (Asn) in raw commodities accounts for the majority of the AA found in foods. Several minor pathways have been described, as well as a major route originating from the early Schiff base of asparagine and carbonyls.

1. Carbonyls + Asparagine $\rightarrow\rightarrow$ acrylamide (AA)

Three potential pathways can be described from the early Schiff base.

1) Formation of an oxazolidin-5-one intermediate, subsequent decarboxylation under relatively mild conditions to a stable azomethine ylide, rearrangement to the corresponding Amadori product and final β -elimination to afford AA. This pathway can be considered marginal (1-5%) *versus* the classical Amadori route. In this case, the rate limiting step is the β -elimination reaction. Other groups have also shown the general applicability of the β -elimination pathway to other amino acids shown (e.g. phenylalanine to styrene, aspartic acid to acrylic acid, etc.).

However, so far no evidence has been provided for the oxazolidin-5-one or azomethine ylide intermediates. Studies conducted on model Amadori compounds (from phenylalanine) show that β -elimination is favored under "dry conditions" compared to aldehyde formation, provided a β -proton is available for the Hofmann-type elimination reaction. It would also be important to study the impact of pH on the rate of the individual reactions, i.e. β -elimination may be favored at higher pH and this could explain why some studies have shown that higher amounts of AA are found when extracting foods at higher pH.

2-3) As depicted in **Figure 1**, decarboxylation of the Schiff base may proceed *via* the zwitter-ionic form leading to the azomethine ylide.

Either a) cleavage of the imine directly to give AA, or b) hydrolysis of the imine to afford 3-amino-propionamide (3-APA), also termed β -alanine amide that is further deaminated to AA. The rate-limiting step of this sequence is the decarboxylation of the Schiff base. The reaction has been shown to be more efficient with glucose. The intermediate

3-APA has so far been identified and quantified in potatoes, and can also be formed by direct decarboxylation of free asparagines (decarboxylase activity of the potato tuber). These studies, conducted by Prof. Schieberle (Muenich) have shown an efficient yield of AA from 3-APA (~ 60%) in high moisture model systems. It should be investigated if this intermediate is involved in the formation of AA under pressure cooking / retorting conditions in certain foods.

2. Aspartate + Sugar $\rightarrow\rightarrow$ acrylic acid + NH_3 \rightarrow acrylamide

The formation of corresponding vinylogous compounds from their amino acid progenitors by a pathway has been shown. Similarly, free aspartic acid in foods could lead to the formation of acrylic acid, that in turn could undergo aminodehydroxylation to AA, provided ammonia is present (thermal reaction). This pathway to AA is considered marginal, and in models equates to about 5% of the efficiency of the asparagines route. Furthermore, addition of asparaginase in a food system (potato flakes) converting asparagine to aspartic acid shows a 97% reduction of acrylamide (see section C.), substantiating that the acrylic acid path is probably minor in foods. Interestingly, it was also shown that β -alanine under pyrolytic conditions is more efficient in generating AA than acrylic acid + NH_3 .

3. Triglycerides $\rightarrow\rightarrow$ acrolein \rightarrow acrylamide

Decomposition of triglycerides (oxidation) can afford acrolein which in turn could react with asparagine to AA via the route described above, acrolein providing essentially the carbonyl source for the Schiff base formation. Alternatively, it could also react with ammonia to afford AA, or to acrylic acid. These pathways are probably of minor importance in the direct formation of AA, providing under certain conditions reactive carbonyls for the Maillard reaction.

Certain "cold pressed" oils can lead to the formation of AA upon heating (e.g. wheat germ), increasing near linearly up to 135°C after a 1h heating period. Reaction probably proceeds as described in Reaction 1 above, provided that asparagine and carbonyls are available to react in the oil.

4. Other

The involvement of several amino acids in the formation of vinyl compounds and other volatile intermediates was studied by pyrolysis and post-pyrolytic on-line derivatization techniques, using Py-GC/MS. β -Alanine undergoes thermal decomposition to afford acrylic acid. Carnosine – that harbors a β -alanine moiety – similarly affords acrylic acid (**Figure 2**). Carnosine can also degrade to AA via β -alanine amide. In fried meat, this may represent a feasible pathway to acrylamide (levels around 20 – 50 $\mu\text{g}/\text{kg}$ detected), and beef typically contains 21 μmoles carnosine /g.

N-methylacrylamide has also been extracted from roasted beef cubes (heated in a domestic oven at 250°C, 30 min). A potential route is acrylic acid + methylamine. Acrylic acid in the presence of creatine also leads to the formation of N-methylacrylamide and N,N-dimethylacrylamide.

Acrylic acid can be formed from serine and cysteine under pyrolytic conditions. This reaction proceeds via pyruvic acid, that can be reduced (e.g. by formic acid) to lactic acid. Dehydration then leads to acrylic acid and AA if an ammonia source is available.

B. Food categories

Focus is on the “Top Eight”, i.e. French fries (Oil Fried), French fries (Oven Baked), breakfast cereals, potato chips, cookies, coffee, toasted bread, pies and cakes. Additional attention should also be given to infant foods, in particular baby foods in jars and teething biscuits. Furthermore, uniquely high products must be taken into account, and research also in these foods encouraged (gingerbread, coffee substitutes, etc.), due to individual-based relatively higher exposure.

C. Methods of mitigation – control/intervention points

Almonds

FDA survey has shown relatively high levels of AA in retail products . Therefore the impact of roasting procedures and conditions were studied in preliminary tests on a number of major varieties.

The amounts of glucose and fructose did not vary greatly. Interestingly, during roasting only a portion of the available free asparagine is consumed, and basically all of the sugars. The formation of AA appears to correlate to free asparagine and color formation (measured in cross-sections of the almonds).

Temperature seemed a more important parameter than time of roasting. Effects of dry versus oil roast and processing methods are beginning to be studied.

Potato chips

Intensive efforts have been placed on controlling acrylamide in potato products, with numerous studies conducted by industry, academia and food control laboratories. The major challenge is scaling up from the test tube to a manufacturing plant, and taking into account the multiple layers of variation that contribute to the overall variability (RSD 15%, 2 σ at approx. 28 %).

Product moisture is one point of processing control that will afford an average relative reduction of AA (0.5% moisture increase equates to approx. 20% reduction of AA). However, care must be taken not to compromise on quality (texture and off- flavor) and shelf-life of the product.

Sugar control can be exercised at the raw commodity stage, and typically a 15 % reduction of reducing sugars provides an equal reduction Too low sugar concentrations, however, will lead to less consumer acceptance of the product. Other measures such as increasing acidity lead to consumer issues (too light color, sour notes).

The most promising approach tested so far under lab scale conditions is the use of the enzyme asparaginase (collaboration with Novozyme, *Aspergillus oryzae*), which in potato flakes (1 h incubation) has resulted in a very significant decrease of AA (97%), retaining acceptable flavor and color in the product. This application has also been tested in French fries, achieving here approx. 80% reduction (after blanching, opening the cell structure). No regulatory issues are foreseen with this application in the USA, but several years are estimated up to industrial applications.

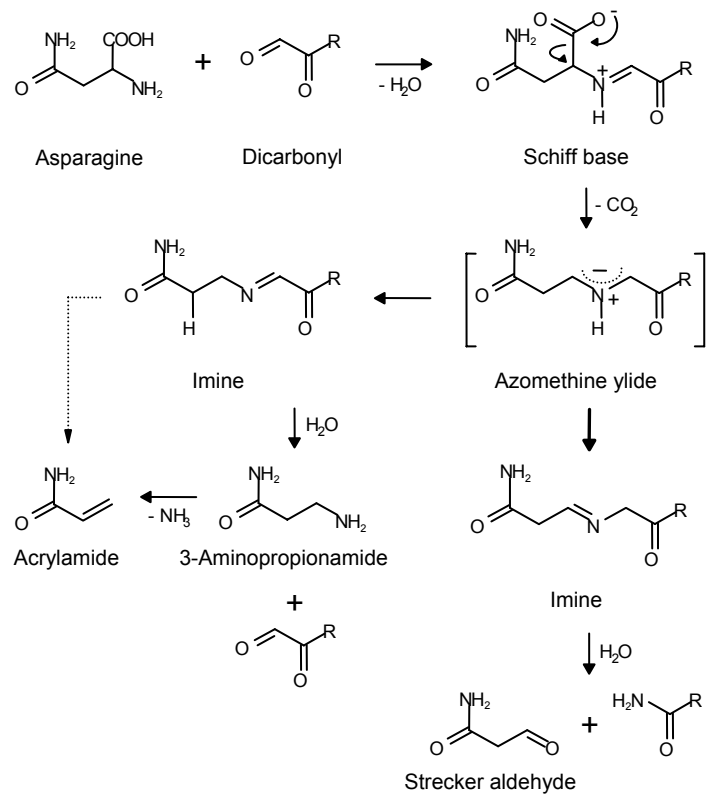


Figure 1. Proposed scheme for the formation of acrylamide by direct decarboxylation of the Schiff base.

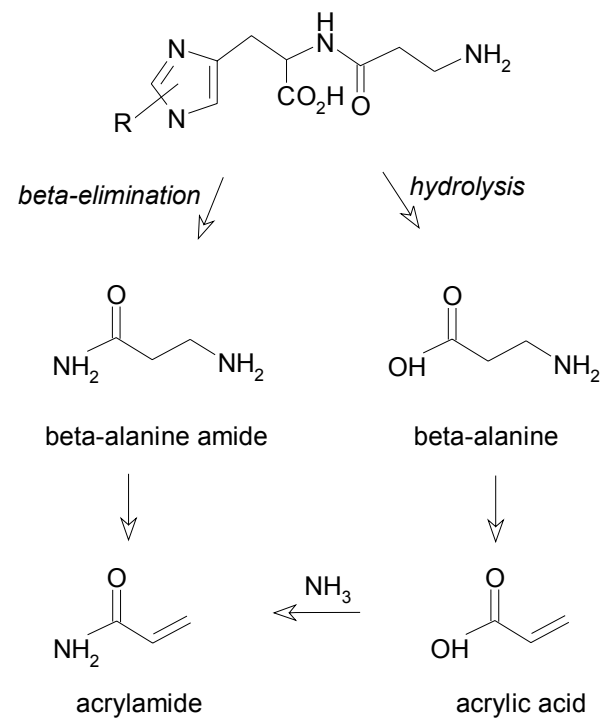


Figure 2. Generation of acrylic acid and AA from carnosine and derivatives.

Potato slices

A project funded by JIFSAN was presented, encompassing general minimization strategies in potato slices. Theoretically, AA can be removed physically (e.g. steam distillation), chemically (self-condensation, reaction with nucleophiles, oxidation). A possibility to convert AA to acrylonitrile was considered using nitrilase (lower b.p. of acrylonitrile).

The experimental studies that were carried out focused on pre-treatments of the potato slices. Dry grilled showed levels 900 – 1038 µg/kg, oil treated and then grilled 330 – 350 µg/kg.

Surface washing afforded a reduction of 28%, citric acid 27%, thiamine 12 %, basil/rosemary/thyme 35%. Blanching (30 s) had no major impact. Competing amino acids (0.5 – 2g/l) were added, and individual amino acids showed no significant difference to surface washing. However, Additive effects were seen, e.g. Glu/Asp provided products with an average of 43% reduction versus controls. The problem of scale up was acknowledged.

Interestingly, at the ACS Anaheim meeting another group (Y. Park) reported reductions of about 50 % with a combination of Ca²⁺ and phytate in French fries. Ca²⁺ and low pH (acidulants) showed about 40% reduction, decreases were not solely attributed to pH.

Breakfast Cereals

A rotary cooked and flaked wheat breakfast cereal was studied, with focus on processing conditions. As shown previously by industrial trials on wheat-based breakfast cereals (CIAA), AA is formed mainly during the toasting step. Pre-drying of the flakes had an impact on AA formation (no linear relationship), but resulted in higher manufacturing losses. Furthermore, a negative effect on flavor was shown. There are no practical means to control flake moisture in the oven, which would provide a potential means of AA control. In the study a large variability within and between lots was observed. Overall, the study showed consensus with CIAA observations so far that there are currently no practical strategies to reduce AA whilst maintaining key sensory attributes. Future work will need to focus on the impact of different wheat varieties.

Coffee

No new knowledge compared to that presented at past meetings, i.e. there exists to date no practical options of reducing AA in roasted coffee. Data was shown during the keynote lecture that AA is not stable in roast & ground coffee (finished product) over longer periods of time. This aspect warrants further study and may open potential avenues of mitigation.

Effects of Consumer food preparation

The percentage uptake of AA due to home preparation is currently largely unknown. The objectives of the study were to investigate the effects of cooking conditions on AA formation, and identifying measures to reduce AA in a home cooking environment.

AA formation was determined in French fries (fried in corn oil), adhering to the manufacturers instructions. Levels of AA increased with frying time and temp. and correlated to the degree of browning (“a” and “L” color values). Soaking of French fries in water for 15 minutes provided a 2/3 reduction in AA levels. Soaking in vinegar had a similar effect (50% reduction, 1:1 vinegar: water). Home storage of potatoes (room temp vs. cold) is also an important factor, which is under the control of the consumer (2-3 fold higher levels in AA observed in refrigerated potatoes).

In bread toasting, the surface temperature of the bread can reach well above 200°C. AA increases with the degree of darkness/toasting. A 2/3 reduction can be achieved by scraping off the surface (1972 µg/kg AA measured in the scrapings).

Factors that still need to be considered are consumer acceptability after introduction of the measures. On the cooking side, the precision of the household fryers is still an open question.

Questions for all Working Groups

1. What have we accomplished so far?

- Importance of free asparagine in the formation of AA in basically all foods so far studied, carbonyls from other sources (not limited to reducing sugars)
- Major route identified (decarboxylation of the Schiff base)
- Role of other amino acids, e.g. carnosine in meat
- Importance of ammonium in certain foods

- Good progress and data on potato crisps and French fries
- Observed aging decline in roast and ground coffee: potential avenues of mitigation

2. What gaps are remaining?

- Identification of the key intermediates
- Quantitation of precursors in foods
- Kinetics, identify rate limiting steps = control points
- Yeast vs. chemical leavening
- Understand formation of AA in closed, high moisture systems (retorted baby foods, prune juice, black olives). Involvement of 3-APA ?
- Alternative route of formation of AA in cooked meat?
- Understand mechanism(s) underlying reduction of AA over time in coffee
- Impact of raw commodities, natural variation, seasonal variation

3. What are the uncertainties?

- Consequences of “slowing down” Maillard reaction, may lead to the formation of less “beneficial” compounds (antimutagens, antioxidants)?
- 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. To study formation mechanisms and kinetics of AA in different foods (potato, cereal/bread, coffee)
- Heatox: need a close interaction in devising mitigation measures and the impact of measures on the formation of other potentially “undesirable” compounds. Will any measures defined cause concern for Heatox?
- How to pass on the message to smaller establishments, caterers, restaurants, retailers with in-store preparation, non-branded products, etc.

5. What research is needed?

- See 2. above
- Advanced glycation end products
- Role of water in the different foods

WG1-Specific Questions

1. What is a realistic reduction?

- Modest reductions have been achieved in Europe in certain food categories, i.e. potato products, crisp bread, baby biscuits. In others, there are considerable hurdles (e.g. breakfast cereals, coffee) with possibly no solutions at hand. The impact of seasonality has so far not been adequately studied.
- Controls at the processing level have had minor effects. No substantial further reduction can be envisaged without compromising quality or fundamental changes in raw materials pretreatment or processing.

2. Does this reduction significantly reduce the exposure to acrylamide from food?

- What reduction is necessary to reduce the overall risk? (Question to WG Toxicology).
- Need a 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.

3. What is a realistic date(s) to achieve the target?

- Several years up to industrialization of the asparaginase solution for 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.
- Measurement of intermediates (retorting, formation in certain foods under high moisture conditions).