Process contaminants in different food products and potential strategies of control

Prague, September 29 to October 1, 2004
A. Studer
Symposium on Chemical Reactions in Foods V

Structure of presentation

- Chemical molecule
- History
- Mitigation
- Actual Situation
- Summary+Next
Figure 1. Standard diets, processing and contaminants.

HAA’s – HPA, NitrosAmine, Oxysterol
HPA; MCPD, Acrylamide, CML
APH; MCPD, Acrylamide, Furan
Acrylamide, Oxysterol-CM, transFA

mutagenic, carcinogenic, pro-inflammatory effects
biological indicators: blood and urinary AGES/
oxidant stress; cytokines and RAGE, DNA damage

HAA’s: Heterocyclic aromatic amines; CM: cyclic monomers; PAH: Polycyclic Aromatic Hydrocarbons; CML: Carboxy-MethylLisine; transFA: trans fatty acids

(June04-Icare project)
Hazard Identification in the Food Supply Chain

- **STORAGE**
  - Migration (BADGE, etc...)
- **FOOD PROCESSING**
  - Cross-contamination
  - Mycotoxins
  - Adulteration
  - e.g. Chloropropanols, Acrylamide, AGEs
- **PACKAGING**
  - Tampering
- **TRANSPORT**
  - Meat, milk, eggs
- **SALE**
  - Agrochemicals, Pesticides
- **COOKING**
  - e.g. HAAs, PAHs, acrylamide, etc...
- **CONSUMPTION**
  - Animal feed
- **FARM ANIMALS**
  - SOIL
Process-Induced Contaminants

-> Food-borne contaminants

1. Chloropropanols
2. Acrylamide and
3. Other vinylogous Maillard degradation compounds
4. Furan
1949  For the first time the monomer, acrylamide, was synthesized.

1950  Acrylamide has been used to manufacture polyacrylamide:
   -> Polyacrylamide contains max. 0.1% of the monomer acrylamide

1997  Tunnel construction workers in Sweden had health problems.
      The phenomena was related to the incomplete polymerization of the
      acrylamide:
      -> It was discovered, that not only exposed workers but also the
         **control group had acrylamide adducts in their blood**.
      Nevertheless no further investigations were done.

2000  E.Tareke et.al. from Sweden (Acrylamide: A Cooking Carinogen?),
      tested rats on adduct building which originates from AA formed.
      The results did lead to the estimation **that AA is associated with cancer risk.**
In Spring 2002, study results in Sweden reported the presence of AA (acrylamide, 2-propenamide) in a range of fried and oven-cooked foods.

These findings have attracted considerable interest world-wide because AA has been classified as "probably carcinogenic to humans" by IARC.

Its formation has been considered by the UK FSA, WHO/FAO, and the EU.
Research was required related to:

**Chemistry**

- Develop reliable methods to cover all pertinent food matrices
- Elucidation of mechanisms of formation in models and food
- Measures to reduce levels in food

**Bioavailability of AA in food**

- Elucidation of the mode of action as a carcinogen
- Investigation of the relationship between dietary intake and formation of glycidamide-DNA adducts
- Analysis of dietary AA intake, exposure biomarkers and disease endpoints
- **Epidemiological studies on cancer** in populations of known light exposure, e.g. occupational exposed workers.
Corporate Communications feedback: Media inquiries in 2003

• The media did not focus their attention too much on acrylamide.

• If any statement was made, the chips industry was mostly concerned.

• In the Swiss press (taken as an example during the meeting), 30 articles appeared on acrylamide, of which only 1 with alarming tomes.

• Many articles prone AA as old as mankind; AA is seen as a "natural poison" and not as industrially produced.

• However, AA can be considered as a "dormant" issue, which could pop up anytime, especially once the toxicological risk assessment from JECFA will be issued (end 2004- beginning 2005).
Signal Values (SV)

• German "Signal Values" (SV), forcing industries to continuously reduce the AA level in food, are still in place.

• These SV have no legal basis, but still put lots of pressure on industries.

• The SV were lowered for some product categories in 2003.

• The German Food Industry association will try to discuss in 2004 with Authorities on the legal basis and applicability of the SV.
## Acrylamide: Regulatory

->observations of seasonal influences, max. value 1000ppb

### Signal Values: Overview

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Signal Values, 17.09.2002 (μg/kg)</th>
<th>Signal Values, 31.01.2003 (μg/kg)</th>
<th>Signal Values, 26.11.2003 (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fine bakery ware of short pastry</td>
<td>800</td>
<td>660</td>
<td>575</td>
</tr>
<tr>
<td>Breakfast cereals</td>
<td>260</td>
<td>260</td>
<td>200</td>
</tr>
<tr>
<td>Coffee, roasted</td>
<td>370</td>
<td>370</td>
<td>370</td>
</tr>
<tr>
<td>Potato crisps</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Crispbread</td>
<td>610</td>
<td>610</td>
<td>610</td>
</tr>
<tr>
<td>French fries, prepared</td>
<td>770</td>
<td>570</td>
<td>570</td>
</tr>
<tr>
<td>Potato fritter, prepared</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Gingerbread and bakery ware containing gingerbread</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Thin almond biscuits</td>
<td>1000</td>
<td>710</td>
<td>710</td>
</tr>
<tr>
<td>Children’s biscuits</td>
<td>n. c.*</td>
<td>n. c.*</td>
<td>360</td>
</tr>
<tr>
<td>Diabetics’ cakes and biscuits</td>
<td>n. c.*</td>
<td>n. c.*</td>
<td>1000</td>
</tr>
<tr>
<td>Coffee extract</td>
<td>n. c.*</td>
<td>n. c.*</td>
<td>1000</td>
</tr>
<tr>
<td>Coffee substitute</td>
<td>n. c.*</td>
<td>n. c.*</td>
<td>1000</td>
</tr>
</tbody>
</table>

* n. c.* not calculated

Date: 26.11.2003
Acrylamide: Stakeholders

Stakeholders

• Food producers, processors, caterers, retailers, consumers, Member states
• Progress on ways to lower levels of acrylamide formed in food:
  • Most findings on fried and baked potato, and cereal products
  • Reducing sugars and asparagine, high temperature, low moisture
  • AA levels can be lowered in some foods

Constraints and consumer expectations

• Product quality/safety and consumer acceptance
• Availability of raw materials
• Line modifications
• Production efficiency/cost
• Legal status of additives and other components
• Changes to preparation instructions (label changes)
Gaps highlighted at the recent EFSA Workshop on Acrylamide Formation in Food (17 Nov. 2003)

- Fundamental Research needed on **mechanistic chemistry outside of the known food systems** and Maillard reaction
- Work on **degradation of acrylamide** in food systems
- **Other precursors** not yet identified?
- **Kinetic modeling** will aid in determining rate limiting steps (potential means for mitigation)
- Map total **free amino acid profiles** in crops and raw materials and study impact on acrylamide formation
CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION: in press

A REVIEW OF ACRYLAMIDE : AN INDUSTRY PERSPECTIVE ON RESEARCH, ANALYSIS, FORMATION & CONTROL

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Email: d.taeymans@ciaa.be
Publications Worldwide:

- Acrylamide in title: 1667 (with duplicates)
- (Acrylamide in title) and (food+toxicity): 279 (with duplicates)

- In 2002 (AA in Ti) and (food + toxicity): 36 (including duplicates)
- In 2003 (AA in Ti) and (food + toxicity): 117 (including duplicates)
- In 2004 (AA in Ti) and (food + toxicity): 17 (including duplicates)
Relevant European multicenter studies:

- **HEATOX (EU FP6)** health risks from heat treated foods (8.4 mio €, 23 labs, 3 years)
- **SAFEFOOD (EU FP6)** food safety, risk assessment, communication
- **COST Action 927** thermally processed foods
- **EPIC** European prospective investigation into cancer and nutrition
- **BMBF Netzwerk** nutrition and health
- **ILSI Europe**
  - **AA Task force** develop risk assessment framework
  - **AA Expert Group** discussion document on human exposure and internal dose assessment
- **Swiss Federal Office of Public Health** nutrition and health, mitigation, analytics
- **JIFSAN workshop co-organization (WG on RC)**
## Acrylamide formation in different food products

<table>
<thead>
<tr>
<th>Product Category</th>
<th>Potential Means of Control</th>
<th>Successes or Failures</th>
<th>Outlook</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato products</td>
<td>Reducing sugars, selection of varieties, storage</td>
<td>Reduction by approx. 15% for potato chips</td>
<td>Seasonal variability not yet determined</td>
</tr>
<tr>
<td>Breakfast Cereals</td>
<td>Crop Varieties, Cooker, Toaster, moisture in the different toaster zones</td>
<td>No successes yet achieved. Any changes have a major impact on the product identity, organoleptic properties</td>
<td>Trials ongoing. More detailed investigation of the crop in progress</td>
</tr>
<tr>
<td>Crisp Bread</td>
<td>Changes in baking profile (T, t)</td>
<td>&gt; 30 % reduction</td>
<td>Ongoing trials, impact of raw materials and enzymes to be studied</td>
</tr>
<tr>
<td>Fine Bakery Wares</td>
<td>Modification of recipes, baking temperature</td>
<td>Moderate success, major impact on product quality and organoleptic properties</td>
<td>Trials ongoing, combination of recipe and processing changes needed</td>
</tr>
</tbody>
</table>
## Acrylamide formation in different food products

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<th>Product Category</th>
<th>Potential Means of Control</th>
<th>Successes or Failures</th>
<th>Outlook</th>
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</thead>
<tbody>
<tr>
<td>Baby biscuits</td>
<td>Recipe and baking adapting regimes</td>
<td>&gt; 50% reduction, but impact on texture, organoleptic properties</td>
<td>Trials ongoing. Different technologies being assessed</td>
</tr>
<tr>
<td>Coffee &amp; Coffee Substitutes</td>
<td>None identified. Asn is main precursor, small variation in amounts (very high temp. may lead to other undesirable compounds)</td>
<td>No success; roasting is a fundamental part of coffee production. Loss of acrylamide over time and temp., but also impacts organoleptic quality</td>
<td>Trials ongoing. Impact of free Asn in green coffee beans not yet clarified</td>
</tr>
</tbody>
</table>
Acrylamide: Progresses

State of progress

• EU co-ordination of activities

• Information exchanged worldwide
  • WHO/FAO Infonet (JIFSAN)
  • Codex Committee on Food Additives and Contaminants
    (Arusha, March 2003 and Rotterdam, March 2004)

• Levels in some food can be lowered

• Most progress in potato and cereal products, complex product ranges, other foods affected

• Acrylamide is a genotoxic carcinogen, safety implications in food remain unclear
• **Industry will continue to lead the science** in the analytics and in understanding how acrylamide is formed in foods *

• Intensive studies are focussed on trial work in the different food categories to establish rate-limiting steps and devise strategies to lower formation*

*CIAA Technical Workshop March 2003, paper in press in “Critical Reviews in Food Science and Nutrition”.  
2nd CIAA Technical Workshop Feb 2004, review paper in preparation*
• Elements adding to the complexity in the different foods must be acknowledged (crop attributes, raw material composition, processing changes / mitigation measures)

• Establish a balanced view and focus on providing the scientific evidence for the risk assessment of acrylamide in foods
Formation of 3-MCPD:

- During storage at room temperature (dried culinary products)
- After Heat treatment (cereal drinks)
• Routine analysis of dried culinary products have revealed that chloropropanols were formed during storage.

• Why？
Routine analysis of dried culinary products have revealed that chloropropanols were formed during storage.

“Allyl alcohol is a thermal decomposition product of alliin [(S)-allyl-L-cystein sulphoxide], a cystein amino acid found in garlic and related species” (Kubec et al.; 1997)
Allyl alcohol is a precursor for MCPD
- Allyl alcohol with HCl, HOCl or NaCl solution are able to form 3-MCPD

Onion as well as garlic powders contain chloropropanols
- Thermally treated garlic powders contain more 3-MCPD than onion powders

Formation of MCPD was induced in model system experiments with onion/garlic powders, NaCl and H₂O:
- Influence of compression of the powders
- Influence of pH and water content

Kinetics of chloropropanol formation are slow and cannot explain the contamination observed (culinary products contain ~4% onion/garlic powder)
Based on model system experiments and on a storage study involving eleven culinary dried products, two mechanisms explaining the formation of chloropropanols at RT or low temperatures were investigated:

- chemical
- enzymatic
Enzymatic pathway
Pure glycerol system, neutral pH

Experiment: 1.25 g glycerol, 1.25 iso-octane/hexane/palm oil, 4g NaCl, 32mg pepper, 1g water, neutral pH, RT

In press: Journal of Agriculture and Food Science (M.C.Robert/Nestlé Research)
Enzymatic pathway

Proposed hypothetical mechanism of formation of chloropropanols involving lipase

\[
\text{Lipase} \quad \text{Fatty acid}
\]

\[
\begin{align*}
\text{Cl}^- & \quad \text{O} \\
\text{R}_2 \text{C} & \quad \text{O} \quad \text{CH} \\
\text{H}_2\text{C} & \quad \text{O} \quad \text{C} \quad \text{R}_3 \\
\end{align*}
\]

\[
\begin{align*}
\text{HO} & \quad \text{R}_1 \\
\text{O} \quad \text{C} & \quad \text{R}_3 \\
\end{align*}
\]

\[
\begin{align*}
\text{H}_2\text{C} & \quad \text{Cl} \\
\text{R}_2 & \quad \text{C} \quad \text{O} \quad \text{CH} \\
\text{H}_2\text{C} & \quad \text{O} \quad \text{C} \quad \text{R}_3 \\
\end{align*}
\]

\[
\begin{align*}
\text{H}_2\text{C} & \quad \text{Cl} \\
\text{HC} & \quad \text{OH} \\
\text{H}_2\text{C} & \quad \text{OH} \\
\text{3-MCPD} \\
\end{align*}
\]
Formation of chloropropanols is influenced by:

- Incubation / storage **temperature**
  - Optimum lies at 35-45°C

- pH
  - Optimum lies between pH **7-8** for most lipases

- **Aw**
  - Enzymes in general are hardly active at \( a_w \) below 0.3

- **Chlorine content**
  - High ionic strength may also be deleterious to the enzyme

- **Substrate (oil / fat)**
  - The specificity of the enzyme for its substrate will determine the reaction rate kinetics and thus the rate of chloropropanol formation
Conclusions 1. (MCPD formation)

- MCPD formation depends on the activity of the lipase, the pH and the Aw of the system.
- Technical lipases, as well as lipases from ingredients seem, in general, to have a preference for the hydrolysis of short-medium chain and saturated fatty acids.
  - Formation of MCPD is higher using RBDO palm oil (and peanuts oil) than with olive and sunflower oils.
- Lipase activities found in ingredients/soups correlate well with 3-MCPD formation observed during storage of culinary dried products.
- Thermal inactivation of lipase activity in ingredients (especially meat extracts) is not efficient.
- MCPD could not be formed from glycerol using lipase.
Enzymatic pathway

→ Lipase activity & specificity plays a predominant role in the formation of MCPD in culinary dehydrated products.

Factors influencing the enzymatic activity are pH, Aw, Cl⁻, temperature and substrate

For more details:

MCPD formation during storage

Avoid products which contain Lipase, such as:

- Native Pepper (take extracts)
- Garlic
- Onion
- Other lipase containing spices
- Meat
Goals and deliverables:

- Establish measures to minimize/suppress formation of chloro-propanols for heat treated food categories
- Understanding the mechanisms of formation of chloropropanols in heat treated products, based primarily on model systems

Project status at Nestlé:

- Ongoing
Significant of different MCPD-acylglycerides in products: Development of an LC-MS/MS determination method

HPLC-ESI-MS of Mono-chloro-mono-acylglycerides

- 1-Stearoyl-3-chloroproanediol (m/z 394) 2.15E4
- 1-Oleoyl-3-chloroproanediol (m/z 392) 5.25E4
- 1-Heptadecanoyl-3-chloroproanediol (m/z 380) 1.94E4
- 1-Palmitoyl-3-chloroproanediol (m/z 366) 1.58E4
- 1-Myristoyl-3-chloroproanediol (m/z 338) 1.55E4
- 1-Lauroyl-3-chloroproanediol (m/z 310) 1.87E4

TIC (3.92E5)

On-going)
Mitigation:

Operational parameter control:

• Temperature
  ➢ as low as possible

• pH control
  ➢ as high as possible
May 7, 2004:

- Health Canada's published on their Web-homepage a study about formation mechanisms.
- Their proposed pathways are (at higher temperatures):
  - Oxidation of polyunsaturated fatty acids
  - Decomposition of ascorbic acid derivatives.

May 10, 2004:

- FDA published (WEB) a survey on Furan data:
  - Found in food products.
  - As well as the analytical method used for Furan determination.
Our group developed an analytical method in order to confirm the published results as quick as possible.

The procedure was chosen considering the following parameters:

- Due to the high volatility of Furan, it was decided to analyze it by headspace connected to GC-MS.

- Furthermore, the use of Solid Phase Micro Extraction (SPME) was chosen as a quick and sensitive extraction method.

- In order to achieve reliable results, an isotopomer (d4-furan) was used as internal standard.
<table>
<thead>
<tr>
<th></th>
<th>FDA</th>
<th>NRC(Nestlé)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Static head space</td>
<td>Static head space</td>
<td>SPME</td>
</tr>
<tr>
<td>Sample size: 5 g</td>
<td>Sample size: 5-50 mg</td>
<td></td>
</tr>
<tr>
<td>Standard addition curve</td>
<td>Standard addition curve</td>
<td>External calibration curve</td>
</tr>
<tr>
<td>Two ions for furan, one for d4-furan</td>
<td>Two ions for furan, one for d4-furan</td>
<td>Three ions for furan, two for d4-furan</td>
</tr>
</tbody>
</table>
FDA (May 2004),
Dr. R. Brackett, director of the FDA:

• Learn more about whether furan, particularly at these very low levels, poses any significant problem to human health.

• It's important to stress that FDA's preliminary estimate of consumer exposure is well below the level that would be expected to cause harmful effects.

• FDA is soliciting information on the best available and most-up-to-date science on furan including human exposure:
  • Why furan forms in certain foods and
  • The effect of furan on humans at the low levels found in food.
### Furan in coffee, source FDA (May 10, 2004)

<table>
<thead>
<tr>
<th>Coffee</th>
<th>Furan (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maxwell House Coffee (brewed)</td>
<td>37.4</td>
</tr>
<tr>
<td>Maxwell House Coffee (brewed)</td>
<td>40.4</td>
</tr>
<tr>
<td>Folgers Classic Roast Coffee Crystals</td>
<td>&lt;2.0</td>
</tr>
<tr>
<td>Folgers French Roast Coffee (brewed)</td>
<td>44.7</td>
</tr>
<tr>
<td>Nescafe Classic Instant Coffee</td>
<td>&lt;2.0</td>
</tr>
<tr>
<td>Nescafe Classic Instant Coffee</td>
<td>&lt;2.0</td>
</tr>
<tr>
<td>Starbucks Yukon Blend whole bean (brewed)</td>
<td>84.2</td>
</tr>
<tr>
<td>Maxwell House Sanka Decaffeinated Coffee (brewed)</td>
<td>33.6</td>
</tr>
<tr>
<td>Maxwell House Sanka Decaffeinated Instant Coffee</td>
<td>&lt;2.0</td>
</tr>
<tr>
<td>Folgers Classic Decaf (brewed)</td>
<td>42.5</td>
</tr>
<tr>
<td>Folgers Classic Decaf (brewed)</td>
<td>38.3</td>
</tr>
<tr>
<td>Folgers Classic Decaf (brewed)</td>
<td>52.6</td>
</tr>
<tr>
<td>Nescafe Taster's Choice Decaffeinated Instant Coffee</td>
<td>4.8</td>
</tr>
<tr>
<td>Nescafe Taster's Choice Decaffeinated Instant Coffee</td>
<td>7.2</td>
</tr>
</tbody>
</table>
After the food advisory committee meeting (June, 2004), and after evaluating all the available data, FDA will decide:

• Appropriate next steps, which may include an expanded food survey

• Studies to address how furan forms in foods

• Potential strategies to reduce furan levels and

• Toxicology studies to address mechanisms of toxicity and dose response.
Conclusions:

• More research investigations should be done in order to carry out serious risk assessment study in food.

• Furan is simply an individual case like acrylamide (Maillard degradation products).

• This needs to be assessed on a wider basis, i.e. risk benefit analysis.
Summary: Process contaminants

3-MCPD’s:
- Formation over storage: Evaluated
- Mitigation: low temperature and pH adjustment
- Formation heat induced: On-going research
- Toxicity: Yes (kidney, infertility by high doses)

Acrylamide:
- Adapting temperature/time profile
- Choosing the particular crops with low precursors
  - In some products, reduction is successful and in some not.
  - Asparagine sometimes the limiting precursor and sometimes red.sugar.
- Acrylamide is the biggest, stormiest political issue in the contaminant history.
- Toxicity: Animal -> (carcinogenic)

Furan:
- Mitigation, not yet as an issue.
- FDA evaluates the follow-up.
- Mitigation: Control temperature, precursor, etc.
- Toxicity: Yes (carcinogenic)