Chloropropanols and their esters

Dr. Ines Baer
Research fellow at the Food Safety & Quality Unit

IRMM - Institute for Reference Materials and Measurements
Geel - Belgium
http://irmm.jrc.ec.europa.eu/

Joint Research Centre (JRC) – European Commission
Context

- International Measurement Evaluation Program (IMEP)


- Review:

  *Baer I, de la Calle B, Taylor P. 3-MCPD in food other than soy sauce or hydrolysed vegetable protein (HVP), Analytical & Bioanalytical Chemistry, 2010, 396 (1), 443-456.*
## Introduction

- Chloropropanols → four major substances

<table>
<thead>
<tr>
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<th>2-MCPD</th>
<th>1,3-DCP</th>
<th>2,3-DCP</th>
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<td><strong>Occurrence</strong></td>
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<td>1,3-dichloropropanol</td>
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![Chemical Structures](attachment:chemical_structures.png)
- **Two of them raised more concern**

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![Chemical structures of 3-MCPD, 2-MCPD, 1,3-DCP, and 2,3-DCP](attachment:image_url)
Introduction

- **First detection in 1970s**
  
  - group of Jan Velíšek in Prague were first to demonstrate that chloropropanol could be formed in hydrolysed vegetable proteins (HVP)
  
  \[ HCl + \text{glycerol / lipids} \rightarrow \text{chloropropanols} \]

- **Focus on HVP and soy sauce because of their high 3-MCPD level**
  
  - soy sauce is traditionally manufactured by enzymatic fermentation of a mixture of soy beans and toasted/cooked wheat flour (up to 18 months)
  
  - process can be speeded up by acid hydrolysis (reflux soy meal and wheat with HCl for 12-16h) but product lacks many of the essential flavours
  
  → mixing of both products and introduction of flavour enhancers such as HVP
Introduction

- Surveys showed that other foods also significantly contribute to 3-MCPD intake
  
  ➢ lower levels but very regular consumption
    • bread
    • noodles
    • meat
    • cakes and biscuits
    • ...

- 3-MCPD intake is difficult to avoid

"WE THANK YOU FOR THIS FOOD AND ASK YOU TO PROTECT US FROM PESTICIDES, ADDITIVES AND PRESERVATIVES."

... and contaminants?
Introduction

1,3-DCP

- main occurrence reported in minced meat, sausages, bread and only then soy and similar sauces
- Food Standards Australia New Zealand found 1,3-DCP in some samples without 3-MCPD → indicating independent formation pathway

3-MCPD in non-food

- key compound: epichlorhydrin
- contained in resin used for paper, coating material production or for water purification

Investigation of toxicity and risks for health
3-MCPD

- shown to affect male fertility, kidney functioning and body weight in rats when given regular high doses
- no significant genotoxic potential in vivo

JECFA (2001) has classified 3-MCPD as a non-genotoxic carcinogen

1,3-DCP

- can be considered as a genotoxic carcinogen, causing cancer by directly damaging genetic material, however at very high levels

Nothing known about the other chloropropanols
Toxicity & Regulations

- Commission Regulation (EC) No 1881/2006 sets the tolerable daily intake (TDI) at 2 µg kg⁻¹ bw (bodyweight)

- European Union set regulatory limit of 0.02 mg kg⁻¹ in HVP and soy sauce (liquid with 40 % dry matter)

- Regulations only for HVP and soy sauce

- No regulation for other food types

- No regulation for 1,3-DCP
  - should be kept as low as possible
  - formation is less clear than for 3-MCPD
Formation pathways

3-MCPD: three main pathways

- Acid hydrolysis
- Heat processing
- Esters
Formation pathways

3-MCPD - Acid hydrolysis

→ acid-HVP production

- Hydrolysis is carried out at 100 °C – 130 °C using 4-6 M HCl for 4 – 24h, followed by neutralisation with NaOH.

- 3-MCPD is formed from the reaction of HCl with residual vegetable oil.

- The main precursors are triacylglycerols, phospholipids and glycerol.
Formation pathways

3-MCPD - Acid hydrolysis

Best described in:


Formation pathways

3-MCPD - Heat processing

- Precursors: lipids and sodium chloride
- Formed during baking, cooking, grilling, etc.
- Free glycerol liberated from the triglycerides reacts with present chlorides
- Formation depends on:
  - water
  - pH
  - temperature
  - other substances present
Formation pathways

3-MCPD - Esters

- contaminant is released from 3-MCPD esters by lipase-catalysed hydrolysis

  - can take place in the body and/or food
  - assuming 100% hydrolysis, 3-MCPD intake would be manifold the TDI

BUT, it seems that:

  - 3-MCPD esters behave like triacyl-sn-glycerols in the gastrointestinal tract
  - gut lipases have a higher affinity for positions sn1 and sn3
  - assuming similar metabolism for 3-MCPD esters would mean that sn1-monoesters (~15%) would result in the release of free 3-MCPD, whereas the sn2-monoester would be absorbed as such
Formation pathways

3-MCPD monoesters

Gut lipase action

Absorption

Figures from http://lipidlibrary.aocs.org/Lipids/simple.html
Cereal derived products

- glycerol accounts for 68% of the 3-MCPD formed during baking
- mainly generated by yeast during proving
- mostly found in the crust → exposure to high temperature
- other substances that can have an impact are: flour, flour improvers, baking agents

- sugar, storage of dough
- synergetic effects
Occurrence

Malt-derived products

- malted grains, malt flours, malt extracts, products used for colouring and flavouring purposes

- 3-MCPD results from the dry-kilning of malted and unmalted barley above 170 °C

- presence is inevitable, but of less importance since substance will be diluted in final product

- BUT apparently high 3-MCPD ester content
Coffee

- can be found in roasted coffee, but highest level in instant coffee
- the final colour of the coffee beans is directly linked to the 3-MCPD formation
- BUT no detection of 3-MCPD in coffee beverages because of dilution with water

Cheese

- can be found in melted or grilled cheese
- exact mechanism of formation not yet known, but it is assumed that abundantly present glycerols and chlorides are responsible
Occurrence

Smoked food

- study in Germany revealed high levels of 3-MCPD in smoked meat, which seemed to be dependant on the duration and the type of wood used for the smoking
- study of a smoking process showed that the 3-MCPD was in the smoke, but not initially in the pellets

- 3-MCPD content in kipper seems to increase with the salt concentration in the brine and the smoking time

- generally 3-MCPD production is associated with “cold smoking” procedure
- nothing has been found concerning the smoking at high temperatures
Occurrence

Meat

- 3-MCPD can be present in cooked meat such as salami, bacon, hamburgers, ...
- formation not understood as there is no direct link to precursors like glycerol
- a study showed that temperatures >100 °C might be necessary, and that “wet” cooking may hinder 3-MCPD formation
- other suggestions are the transfer from the coating (e.g. for salami) and release from esters
Occurrence

Salted fish

- 3-MCPD was detected in anchovy fillets
- not formed during maturation, but rather during packaging and storing
- again formation is not clear, but may result from enzyme action on fats
  - possible release of glycerol type precursors reacting with present chlorides
  - or chloroesters may be formed, creating 3-MCPD upon hydrolysis
- it is suspected that this type of fat / enzyme-related mechanism might be widespread, but nothing can be found in scientific literature so far
The amount of 3-MCPD is the result of the mass balance between formation and decomposition

- mitigation not an easy task as formation mechanism are not fully known for all types of foods and it does vary

- contaminant results from required ingredients
  - a compromise must be found between sensory characteristics and levels of contaminant-producing ingredients

- microbiological stability and nutritional properties must remain optimal and inadvertent formation of new contaminants avoided

For example - reducing free 3-MCPD in bakery products by increasing the pH-value of the raw material, might result in an increased formation of acrylamide, another undesired contaminant.
Mitigation

Key process parameters:

- pH
- Moisture
- Time
- Temperature

Kinetic models including these parameters would be useful for devising potential reduction strategies.
Mitigation

Possible mechanisms to limit the amount of 3-MCPD:

- Raising the pH of high moisture content food
- Lowering the maximum processing temperature and salt content of the food
- Limiting the amount of glycerol in the food produced during preparation and storage
- Avoiding low water / high temperature treatments
Mitigation

Possible mechanisms to limit the amount of 3-MCPD:

- Avoiding the use of partial glycerides as additives
- Use of spice extract in place of native spices, or reducing the microbial load via thermal treatment
- Confirming the purity of food additives
- Inactivation of lipases / esterases
- Screening food contact materials for 3-MCPD precursors
Some examples:

- Use of micro-organisms or enzymes with dehalogenating properties
  - limitations: non-food-grade material, certain models are oversimplified

- Producing HVP under hydrolysis conditions reducing the 3-MCPD content would affect organoleptic properties
  - proposed alternative: acid-hydrolysed winged bean and soybean proteins with an additional alkaline thermal treatment

- 3-MCPD is unstable under neutral or alkaline conditions
  - use food additives to lower 3-MCPD level
  - when tested most active inhibitor was sodium bicarbonate, followed by sodium carbonate, cysteine and glutathione
Esters

3-MCPD esters

- amount of esters exceeds often by far the 3-MCPD content

Esters

- risk assessment not possible at the moment

- toxicity on their own?
- even if not toxic, do they act as reservoir for 3-MCPD?
- data suggest possible influence of food matrix on the extent of ester hydrolysis → esters in bread were found to be more accessible to gut lipases than esters in pure oils

- formation appears similar to 3-MCPD

- they occur in foods processed at high temperatures, with low water content, high levels of sodium chloride and stored for long periods
- very high occurrence of esters in refined vegetable oils

  - heat treatment and refining process are at the origin
  - esters are probably formed during deodorisation

- three levels can be defined:

  - **Low** level (0.5 - 1.5 mg kg⁻¹): rapeseed, soybean, coconut, sunflower oil
  - **Medium** level (1.5 - 4 mg kg⁻¹): safflower, groundnut, corn, olive, cottonseed, rice bran oil
  - **High** level (>4 mg kg⁻¹): hydrogenated fats, palm oil, solid frying fats

Deodorisation

- **Purpose:**
  - reduce the level of free fatty acids (in case of no previous chemical refining step)
  - remove odours, off-flavours and other volatile components such as pesticides and light polycyclic aromatic hydrocarbons.

- **How:**
  - process is carried out under vacuum (0.5 – 8 mbar)
  - temperatures between 180 °C - 270 °C
  - stripping media: steam or nitrogen

Conditions are adapted within these ranges as appropriate to ensure the removal of specific substances. Further removal of the proteins is achieved at this step.

Esters

Some formation occurs during bleaching.

No significant effects on 3-MCPD formation were observed by increasing the chloride content of the bleaching earth, the use of active carbon, or the fatty acid level at the start of deodorisation.

- no esters detected in virgin seed and olive oil, or in animal fats

- refined oils are often used in tinned fish, antipasti, pesto sauces, etc.

- highly refined vegetable oils and fats are base products for chip fat and margarines

  - highest ester content observed so far
  - probable reason is that these fats undergo the refining process twice
  - additionally, palm oil has a mono- and diglyceride content, which are important precursors
Possibilities to remove 3-MCPD esters and related compounds from processed oils

- Decomposition of formed 3-MCPD esters and removal by steam distillation
- Offering reactant for chloride and removal of the formed volatile product during deodorization
- Enzymatic degradation of 3-MCPD esters and related compounds → reduction of 3-MCPD esters and related compounds
- Treatment of refined products with adsorbents

Knowledge gaps

3-MCPD

- formation is not known for all foods
- toxicology studies

Other

- 1,3-DCP is more toxic, but not much is known about its formation
  ➢ by-product / precursor of 3-MCPD ?
  ➢ independent pathway ?

- other chloropropanols

- esters : metabolism, toxicity, mitigation

- and probably more ...
Thank you!

Cook for 3 minutes stir, wait 30 years to discover if the contaminated ingredient gives you stomach cancer.

inges.baer@ec.europa.eu