PROCESS-INDUCED TOXICANTS IN FOOD

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Toronto 2012

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How process-induced toxicants are formed in food?

1. Introduction

2. Acrylamide

3. Furan

4. 3-MCPD and its esters

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What are process-induced toxicants ?

also known as thermally-generated toxicants

"Chemicals that are formed in food as a result of food processing/preparation that are considered to exert adverse toxicological effects or create a potential or real risks to humans"

From "Process-induced food toxicants – occurrence, formation, mitigation and health risks"
Richard H . Stadler and David R. Lineback (Eds.)
Wiley 2009

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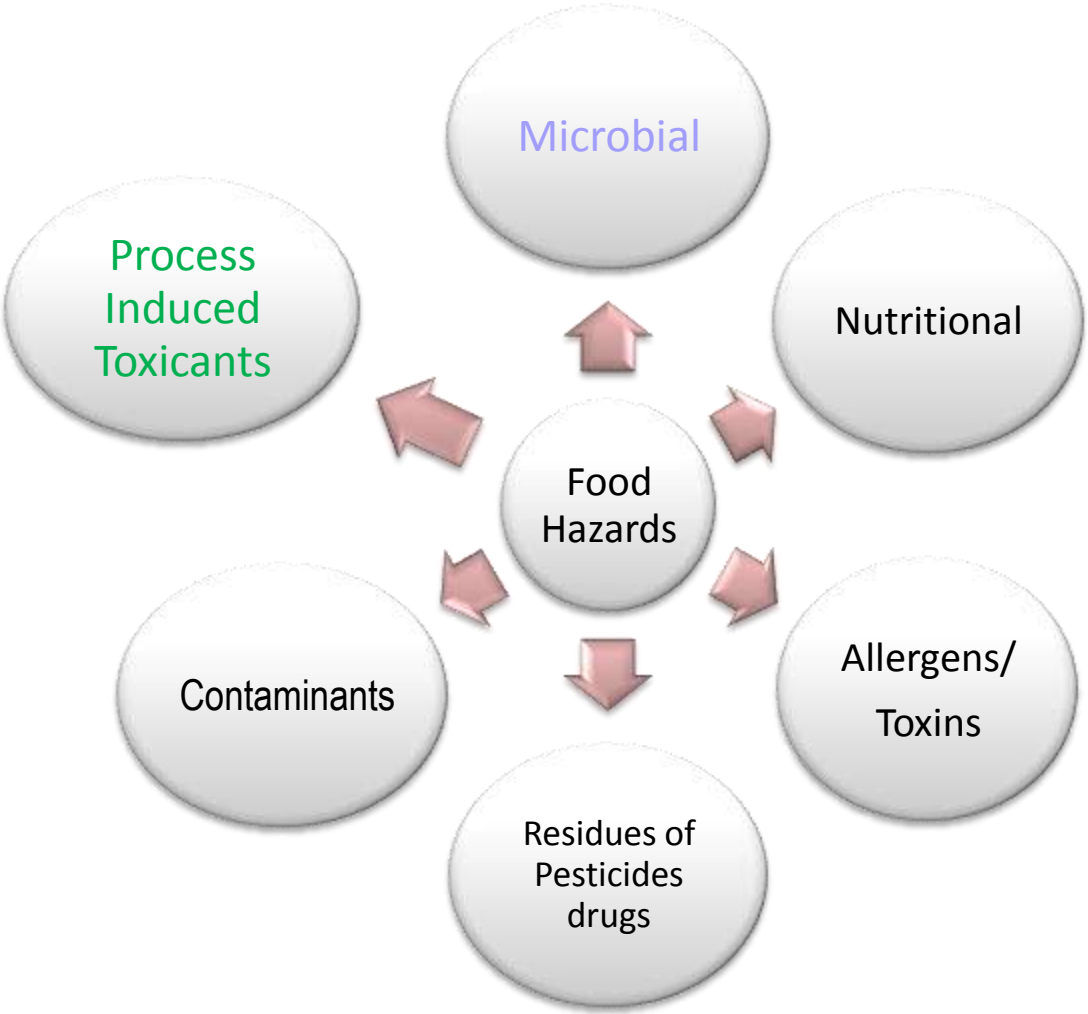
Microbial and Chemical Hazards in Food



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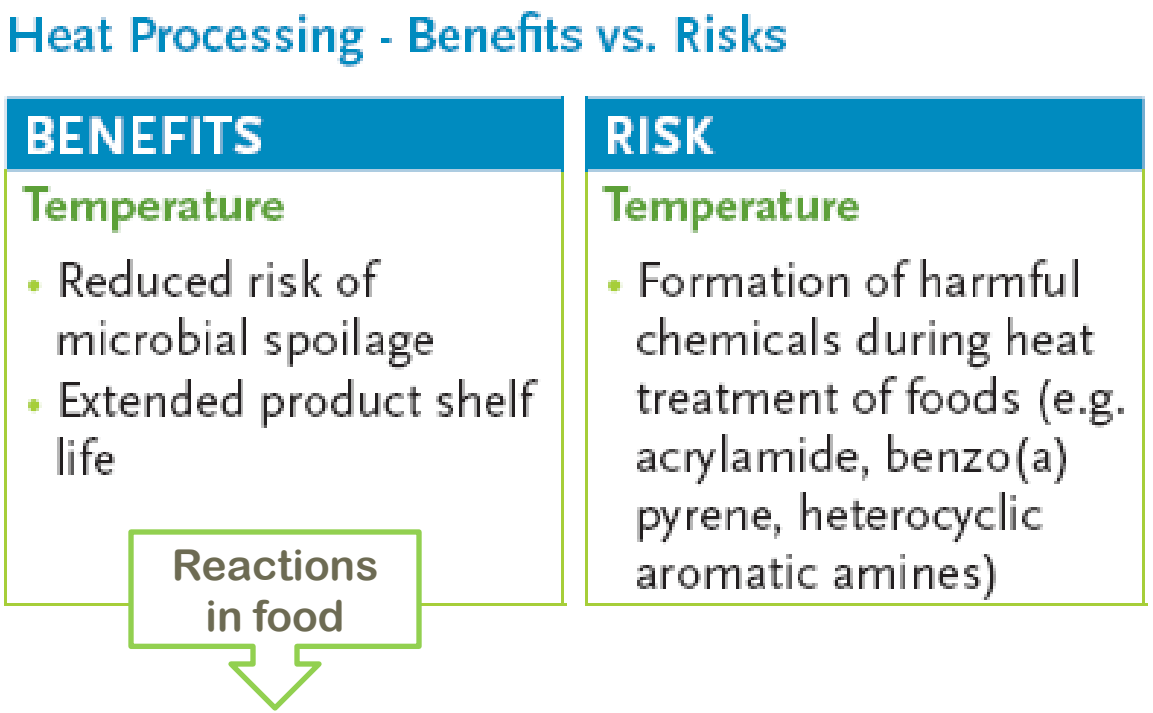
Process-induced or thermally-generated Toxicants



Maillard reaction (color, aroma, taste and texture)
Decomposition reactions of sugars and amino acids
Thermal oxidation of lipids

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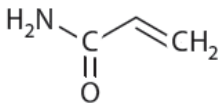
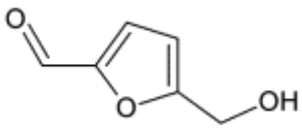
Thermally generated toxicants in food

ppb



ppb

ppm

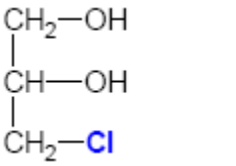


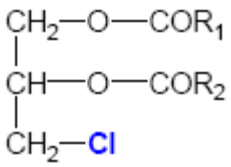
Processed food

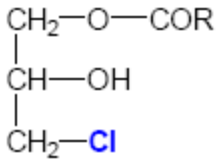
- Acrylamide
- Furan
- HMF
- HAA
- PAH

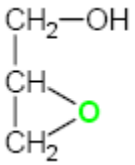
Refined oils

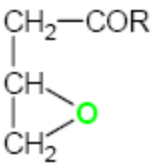
- 3-MCPD
- MCPD esters
- Glycidol
- Glycidol esters











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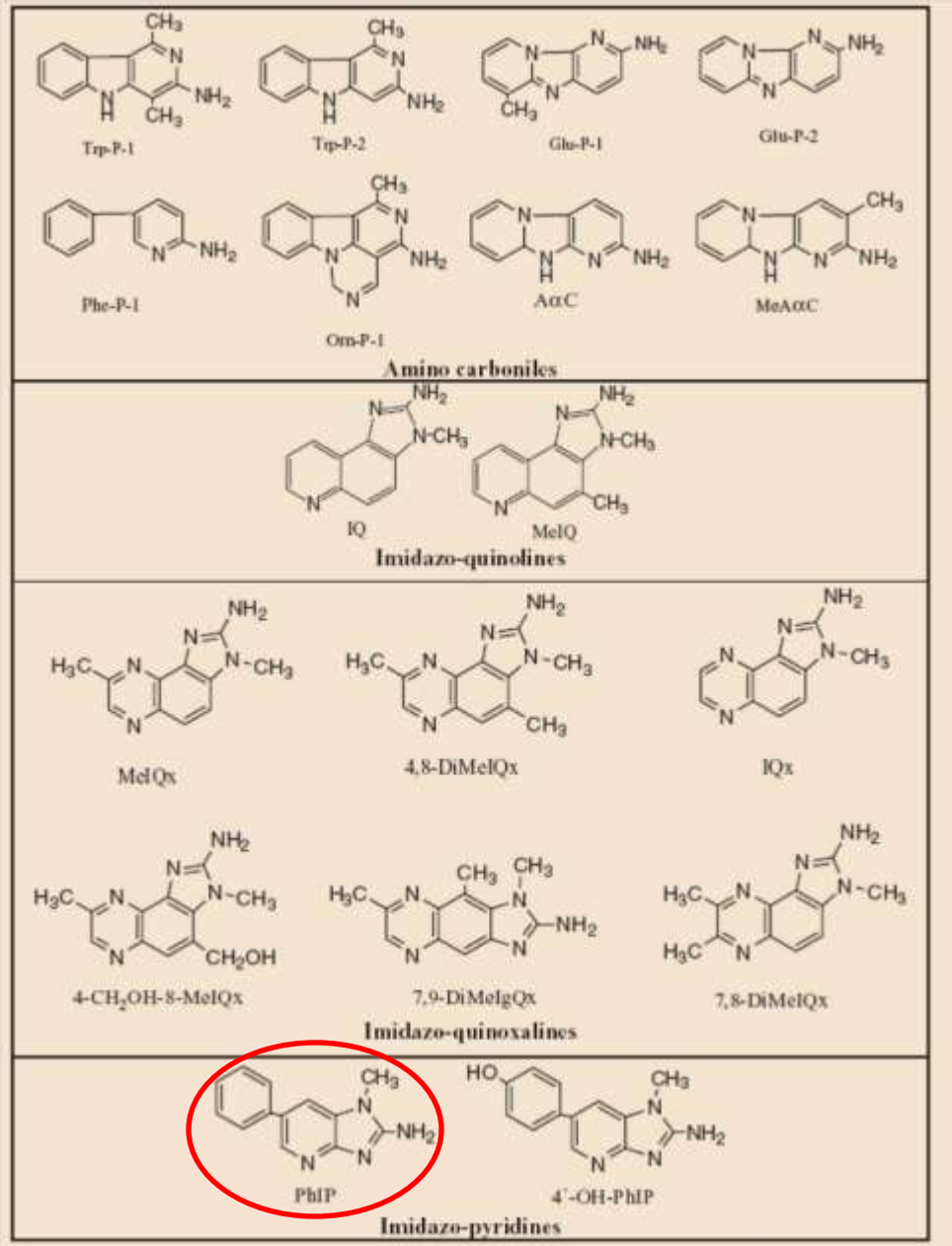

Heterocyclic Aromatic Amines (HAA)

in vitro mutagens
carcinogens
genotoxic to mammalian cells

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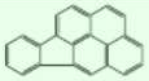
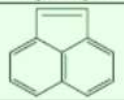
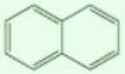
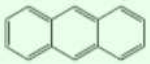
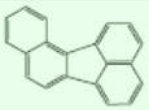
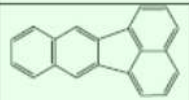
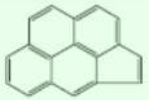
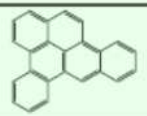
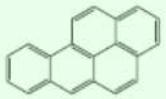
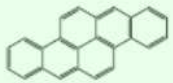
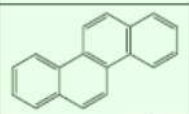
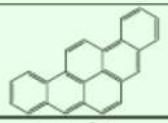
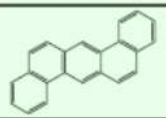
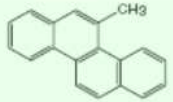
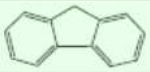
Acenaphthene[a]		Indeno[1,2,3-cd]pyrene[a,b]	
Acenaphthylene[a]		Naphthalene[a]	
Anthracene[a]		Phenanthrene[a]	
Benz[a]anthracene[a,b]		Pyrene[a]	
Benzo[b]fluoranthene[a,b]		Benzo[j]fluoranthene[b]	
Benzo[k]fluoranthene[a,b]		Cyclopenta[cd]pyrene[b]	
Benzo[ghi]perylene[a,b]		Dibenzo[a,e]pyrene[b]	
Benzo[a]pyrene[a,b]		Dibenzo[a,h]pyrene[b]	
Chrysene[a,b]		Dibenzo[a,i]pyrene[b]	
Dibenzo[a,h]anthracene[a,b]		Dibenzo[a,l]pyrene[b]	
Fluoranthene[a]		5-Methylchrysene[b]	
Fluorene[a]			

[a]PAHs in foods identified as high priority by the U.S. Environmental Protection Agency
[b]PAHs in foods identified as of high concern by the European Union

Polycyclic Aromatic Hydrocarbons (PAH)

Mainly carcinogenic

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More than fifty additional thermally generated compounds currently are considered as candidates for possible health hazards based on QSAR studies

an emerging issue in food safety?

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Knowledge Gaps

Knowledge is, in general, lacking on how to effectively deal with thermally generated toxicants. However, in terms of microbiological and allergen risks, risk management is relatively well controlled in an industrial setting through the use of HACCP (Hazard Analysis Critical Control Point) procedures..

It is not known at what levels does the presence of these toxicants in foods become a potential health problem?

Answering such a question normally involves carrying out a quantitative risk assessment.

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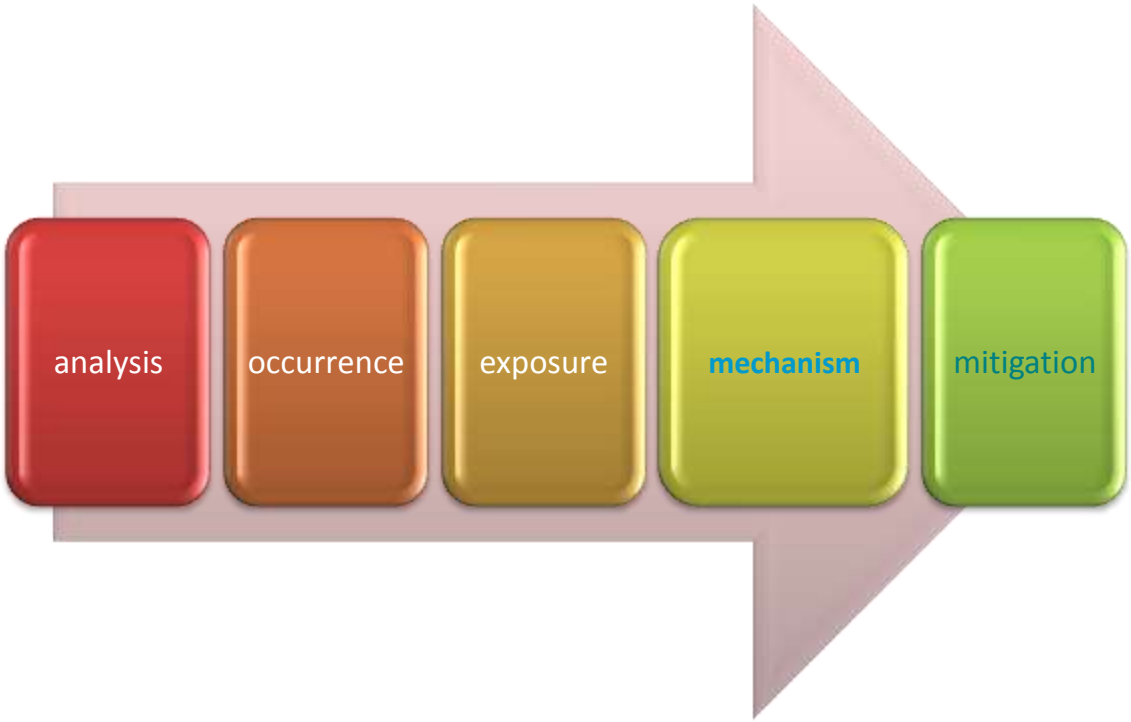
Critical research needs:

Information regarding the five aspects listed below of thermally generated toxicants is critical to accomplishing a quantitative risk assessment that will indicate the likelihood of occurrence of a potentially adverse health effect.



Quantitative Risk Assessment

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Strategies to solve mechanistic problems of process-induced toxicants

Methodologies should be fast and effective to identify the precursors and confirm the mechanism through isotope labeling technique

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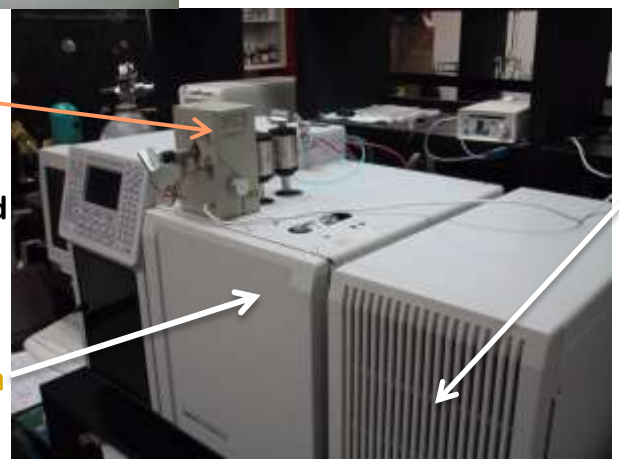
Mechanistic Studies

(1) Based on the structure predict possible precursors that can generate the target hazard from known food components through known chemical transformations if not propose new pathways

(2) Test the hypothesis by performing fast screening through integrated reaction/separation/identification system using thermal desorption units suitable for micro-scale reactions

(3) Repeat using precursors labeled with stable isotopes such as ^{13}C and ^{15}N

(1) Reactants 1- 5 mg (sample preparation 5 min)



(2) Reactor (reaction time 5 seconds) Can be heated up to 800°C

(3) Separation (45 min)

(4) Identification by MS

FTIR with heated ATR crystal to monitor formation of reactive intermediates

Temperature range: ambient to 120°C

Sample size 1-2 mg

Reaction phase: liquid or solid

Total time < 1h

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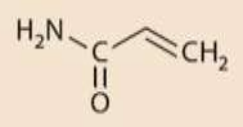
Acrylamide



Using high-dose animal studies, primarily rodents, acrylamide has been found to be a carcinogen and a genotoxin. It is also a neurotoxin in humans. Primarily on the basis of the animal studies, the International Agency for Research on Cancer (IARC) has classified acrylamide as Group 2A – "probably carcinogenic to humans".

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Acrylamide can be formed in almost any food exposed to >120°C and contains asparagine

Summary of reported amounts of acrylamide in different products and product groups.

Product/product group	Acrylamide range (μg kg^{-1})
Potatoes (raw)	<10–<50
Potato chips/crisps	117–4,215
French fries/chips	59–5,200
Bakery products and biscuits	18–3,324
Breads	<10–3,200
Bread (toast)	25–1,430
Breakfast cereals	<10–1,649
Other fruit and vegetable products	<10–70
Chocolate products	<2–826
Roasted coffee	45–935
Coffee substitute	80–5,399
Coffee extract/powder	87–1,188
Meats	<10–116
Dairy products	<10–100
Baby food and infant formula	<10–130

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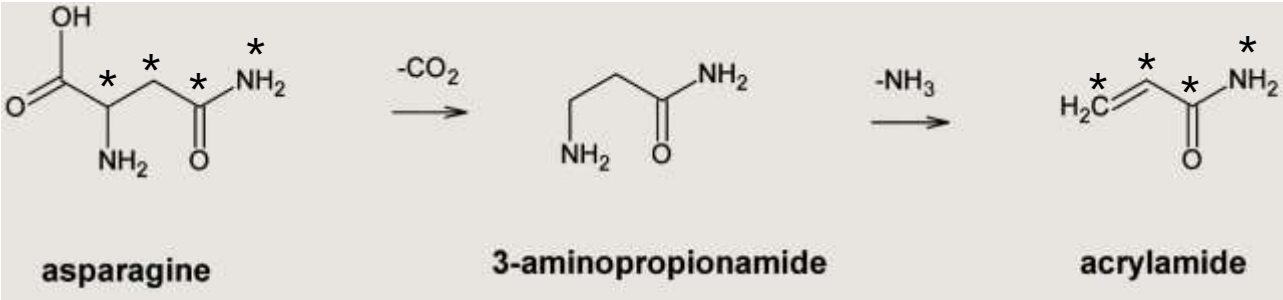
How acrylamide is formed in food?



* Isotope labeled atoms

Isotope labeling studies/mass spectrometry: important tool

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J. Agric. Food Chem., Vol. 51, No. 6, 2003

Table 1. Formation Efficiency of Acrylamide Expressed as Gas Chromatographic Peak Area per Mole of Starting Asparagine Generated at 350 °C from Different Model Systems

model system	efficiency (area/mol)
asparagine alone	0
asparagine + sorbitol	0
asparagine + 2,3-pentanedione	trace
asparagine + glucose	4.9×10^{11}
asparagine + glucose[a]	1.4×10^{11}
asparagine + glycolaldehyde[a]	2.8×10^{11}
asparagine + fructose	6.6×10^{11}
asparagine + glyceraldehyde	8.6×10^{11}
asparagine + sucrose	18×10^{11}

[a] At 250 °C.

Yaylayan et al., J. Agric. Food Chem. **2003**, 51, 1753-1757

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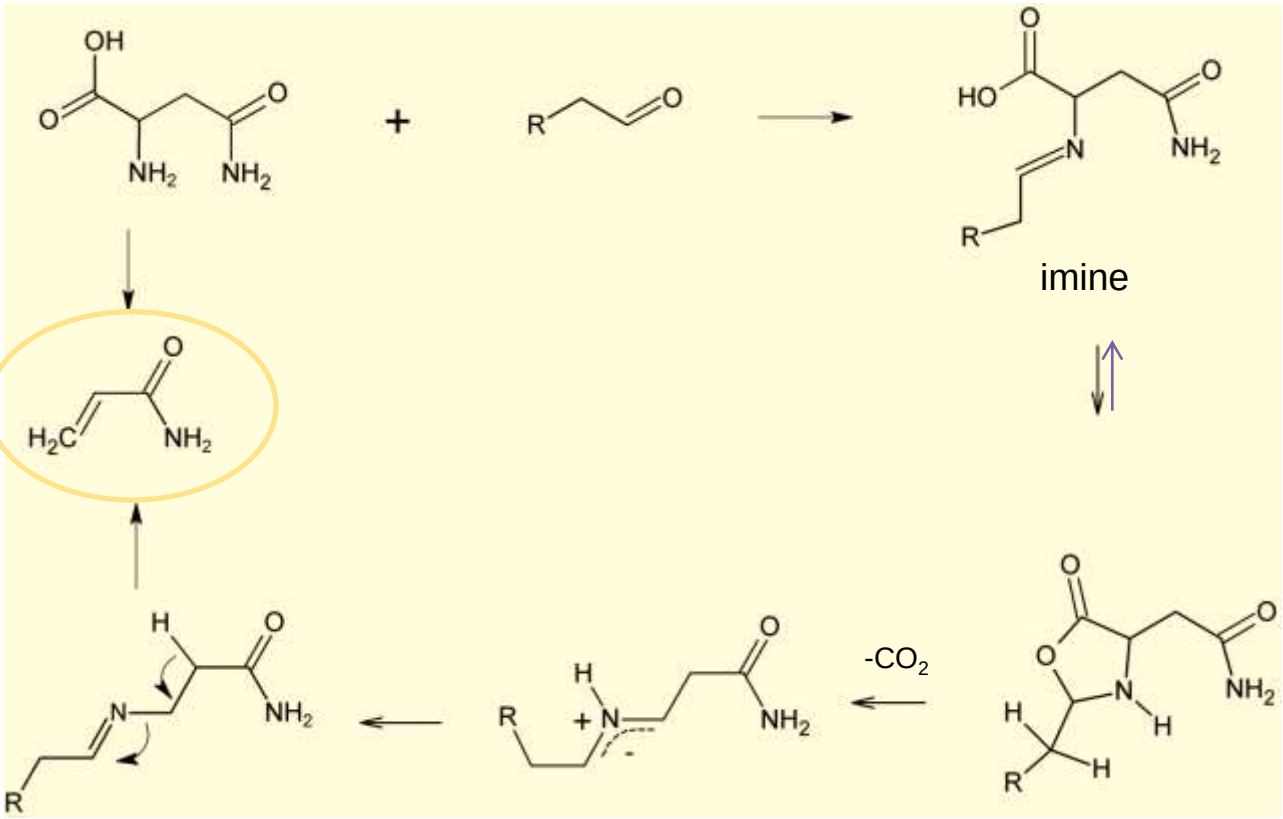
Why do we need sugars?



Yaylayan et al., J. Agric. Food Chem. **2003**, 51, 1753-1757
Perez and Yaylayan, **2008**, 56, 6069-6074

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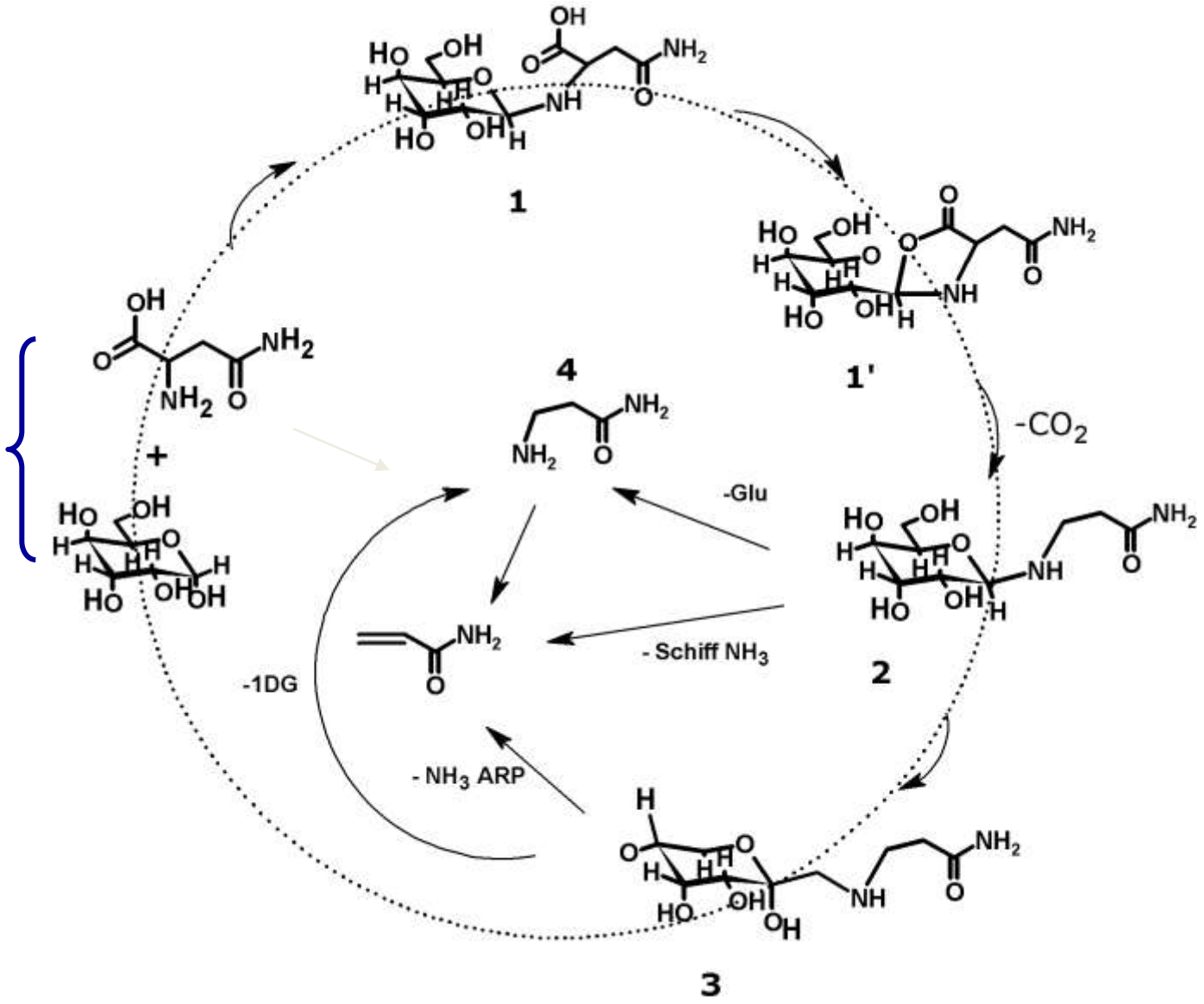
Asparagine
sugar



Perez and Yaylayan, 2008, 56, 6069-6074

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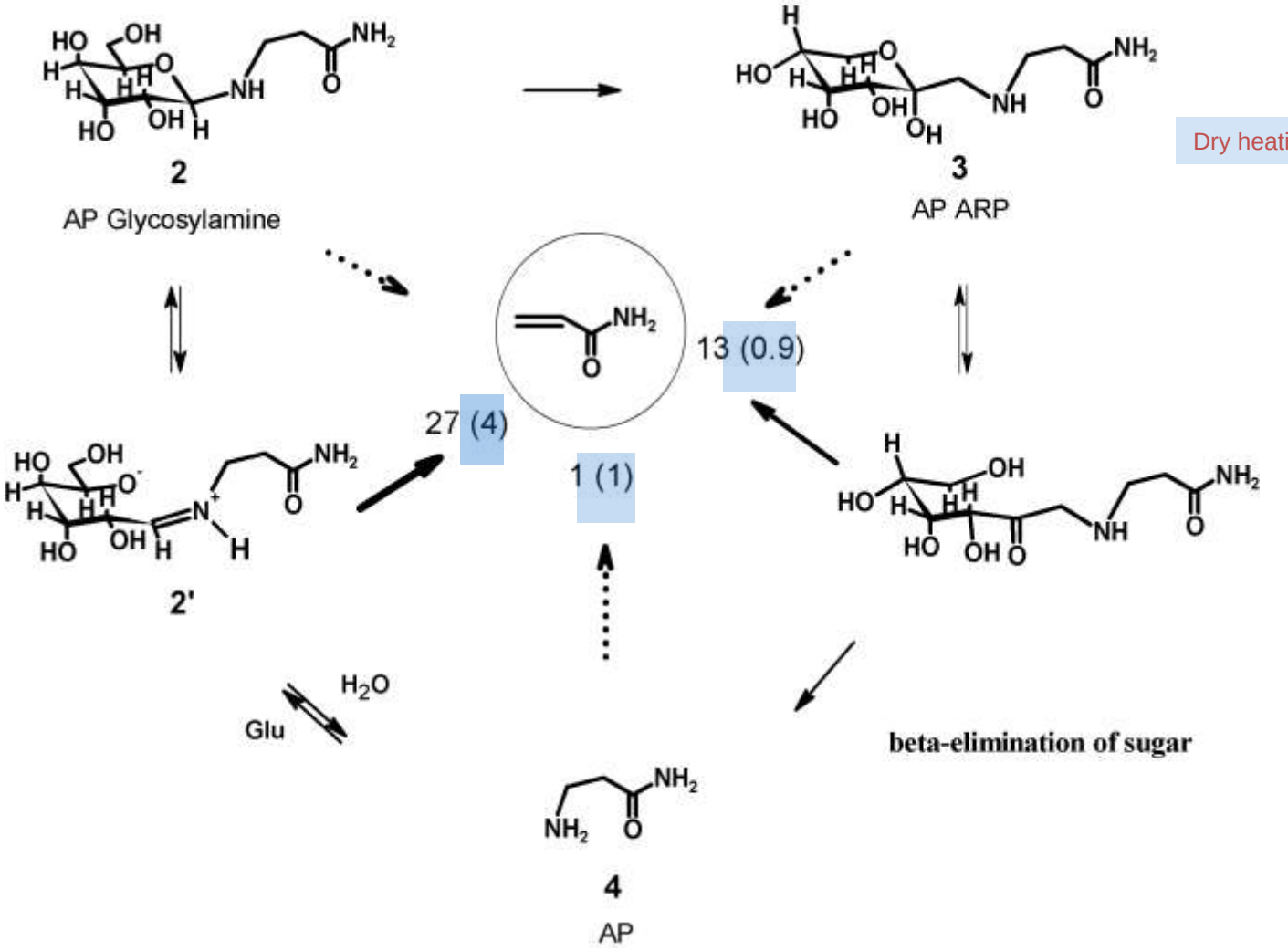
Intrinsic abilities of different precursors to generate acrylamide



Perez and Yaylayan, **2008**, 56, 6069-6074

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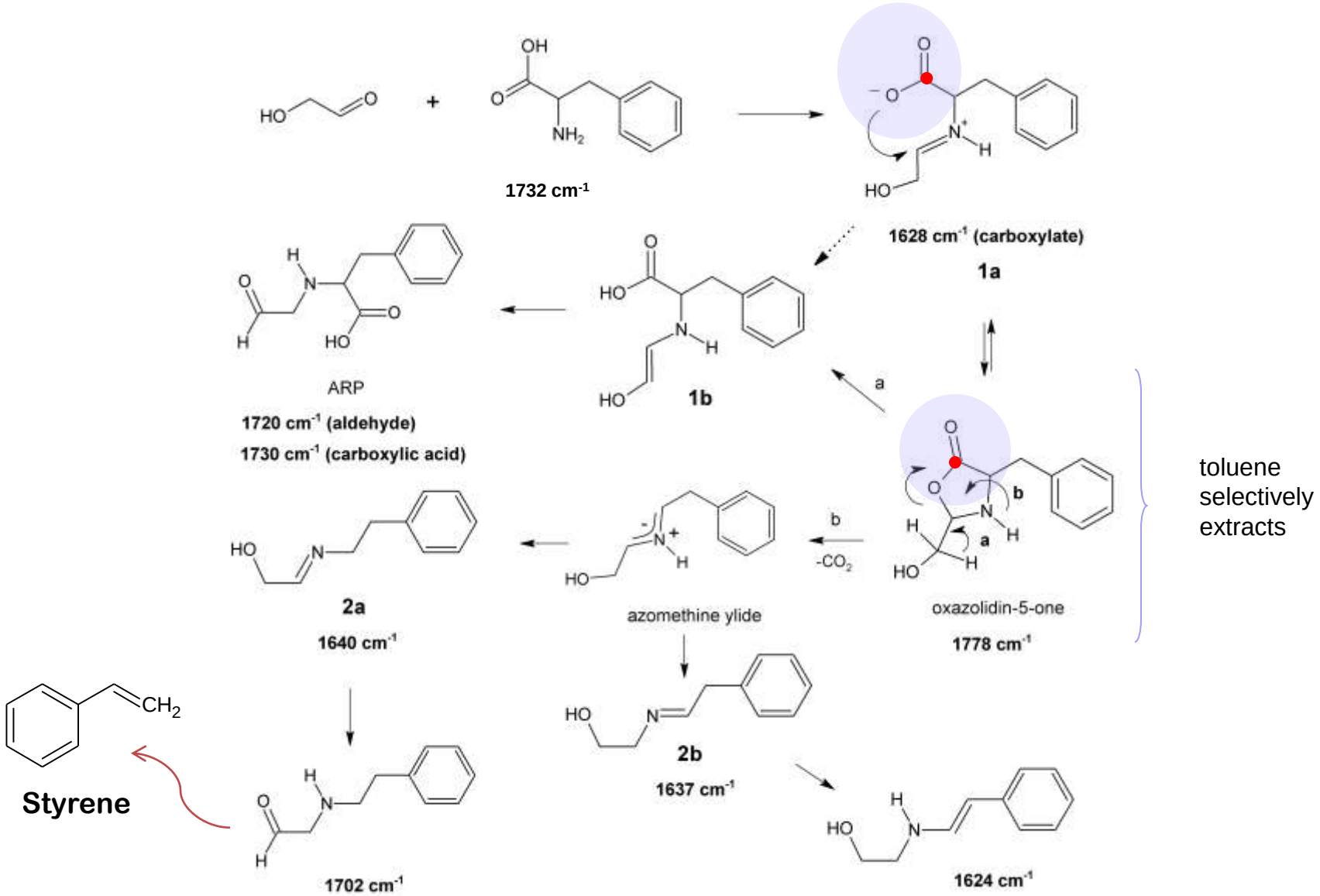
How the formation of oxazolidin-5-one was confirmed?



Chu and Yaylayan, Carbohydrate Research, 2009, 344, 229-236.

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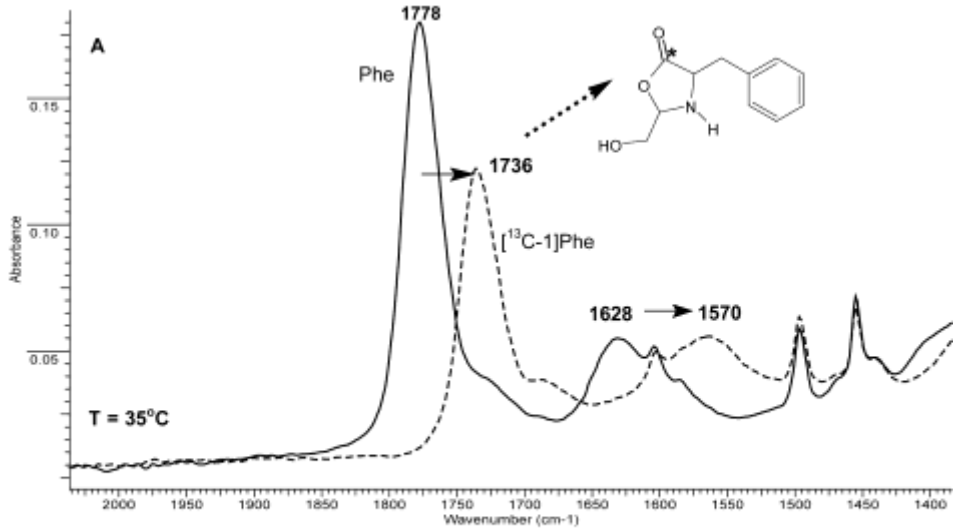
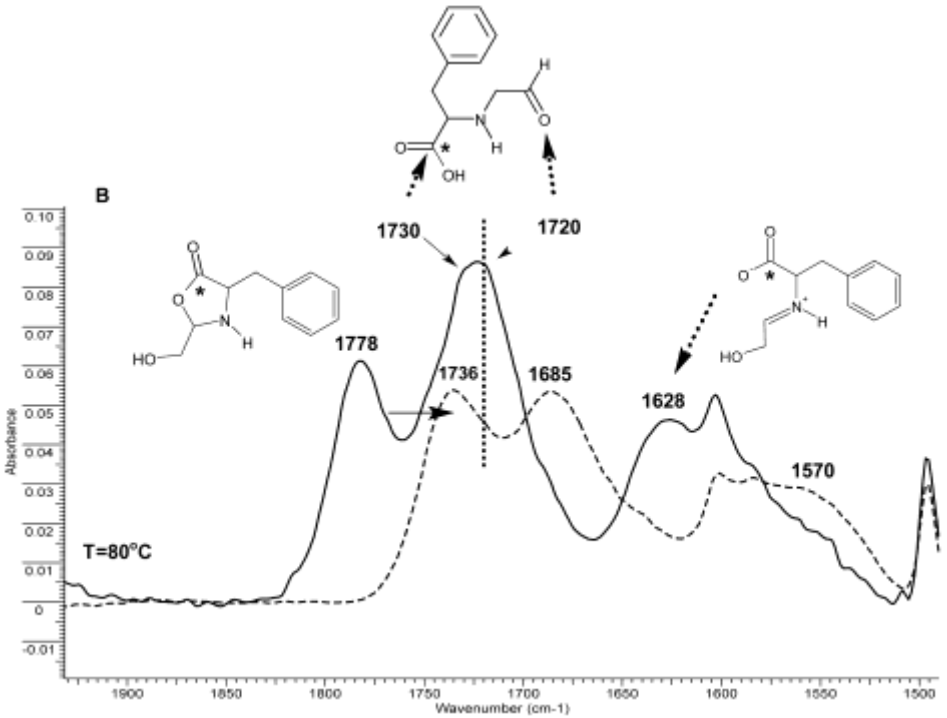
Extraction and reversibility of oxazolidin-5-one



toluene extract of the reaction mixture cooled to 35°C



The same extract heated on the ATR crystal to 80°C

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Mitigation strategies

Depends on food category, (potato products, cereal products, coffee etc.)

Removal of sugars (potato has less sugars than asparagine)
Storage temperature, cultivar effects sugar concentrations in potato

Removal of Asparagine (cereals have more sugar than asparagine)

Adding competing amino acids such as glycine

Adding asparaginase

Lowering the temperature and reducing the time of processing

Removal of sugars and amino acids will also modify color, aroma, texture and taste of the final product through the Maillard reaction

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FURAN

Classified as possible carcinogen to humans (IARC)

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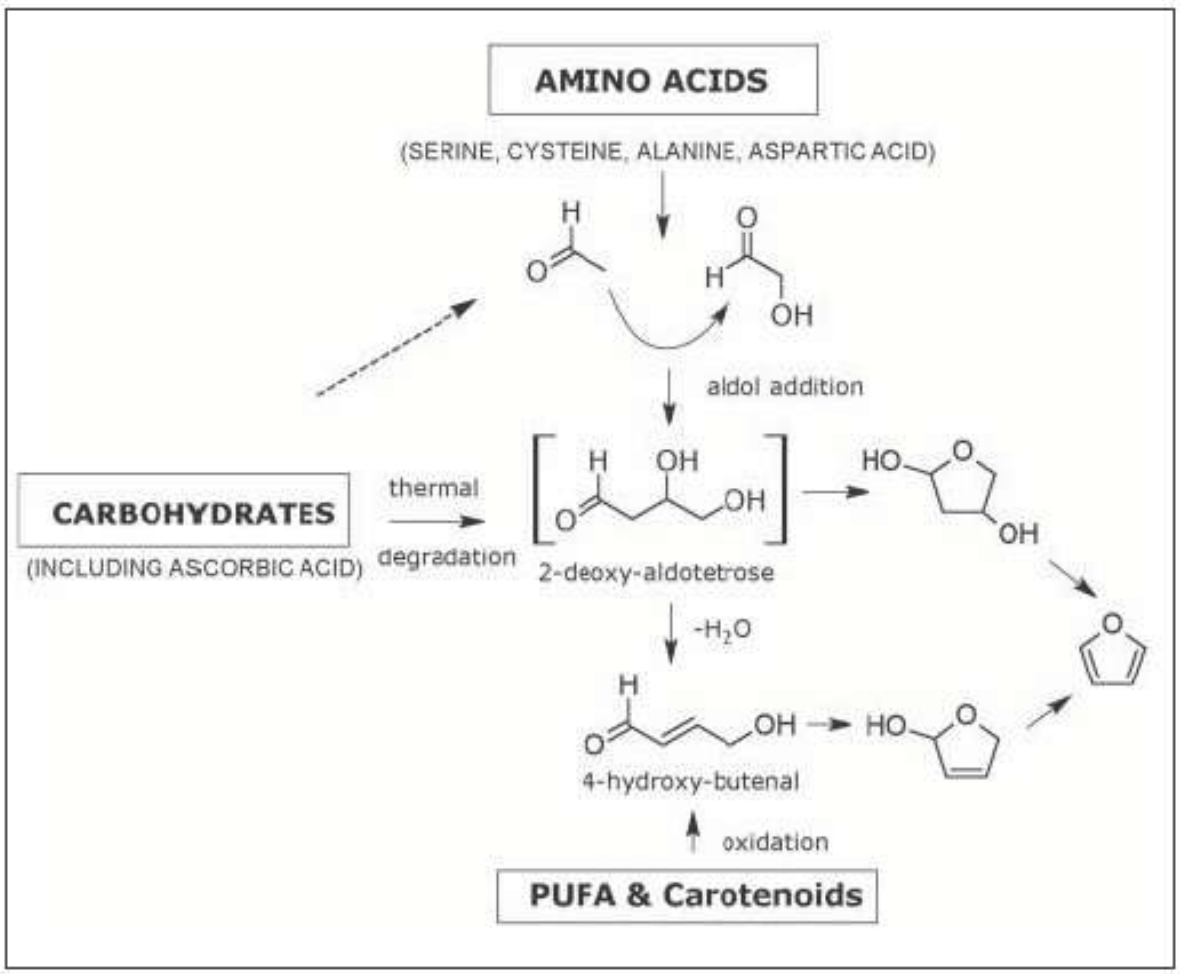
Table 1. Furan concentrations found in some foods commodities on the Swiss Market (according to REINHARD *et al.* 2004 and Swiss Federal Office of Public Health)

Sample description	Furan value (PPB)		Median (PPB)	Number of samples
	minimum	maximum		
Baby food in small glass jars	1	153	12	102
Fruit and vegetable juices for babies and young children	1	40	3	4
Coffee (drink)	13	146	74	9
Hot chocolate and malt beverage	< 2	< 2		2
Canned or jarred vegetables	< 2	12	3	15
Canned soups	19	43		2
Canned fruits	< 1	6		2
Tin containing meat	4	4		1
Tin containing meat and pasta	14	14		1
Sugo, tomato and Chilli sauces	< 4	39	6	13
Soy sauce, hydrolysed vegetable protein	18	91	50	7
Vegetables, fresh	< 1	< 2	< 1	7
Bread and toast	< 2	30	< 2	7
Whole milk UHT	< 0.5	< 0.5		1
Plum beverage	6	6		1
Beetroot juice with fruit juices (organic)	1	1		1
Potato flakes for mashed potatoes (flakes, not prepared)	< 5	< 5		1

Three main precursors: lipids, amino acids and carbohydrates



Yaylayan, V. *Journal of Consumer Protection and Food Safety* **2006**, 1, 5-9.

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Table 1. Relative Efficiency of Furan Formation Expressed as Area Count of Furan per Mole of Starting Material Generated from Different Model Systems at 250 °C

model system	rel efficiency × 10^{10} (area/mol)
L-ascorbic acid	140
dehydroascorbic acid[a]	78
dehydroascorbic acid[b]	47
D-erythrose	32
dehydroascorbic acid	8
D-ribose	7
D-sucrose	5
D-glucose	4
D-fructose	4
L-serine	1
L-cysteine	0.5
L-threonine	0
L-aspartic acid	0
L-alanine	0
L-glycine	0
D-ribose/serine	28
D-sucrose/serine	24
D-fructose/serine	16
D-glucose/cysteine	15
D-glucose/serine	11
D-glucose/alanine	10
D-glucose/L-aspartic acid	9
D-glucose/formate sodium	9
D-glucose/L-threonine	5
D-glucose/glycine	5
D-erythrose/L-serine	3
glycolaldehyde[c]/L-alanine	65
glycolaldehyde[c]/L-serine	9
acetaldehyde[d]/glycolaldehyde[c]	7
acetaldehyde[d]/L-serine	0.5
L-serine/L-alanine	0.4

[a] Pyrolysis temperature = 350 °C. [b] Pyrolysis temperature = 300 °C. [c] From glycolaldehyde dimer. [d] From acetaldehyde diethyl acetal.

Lipids as precursors of furan

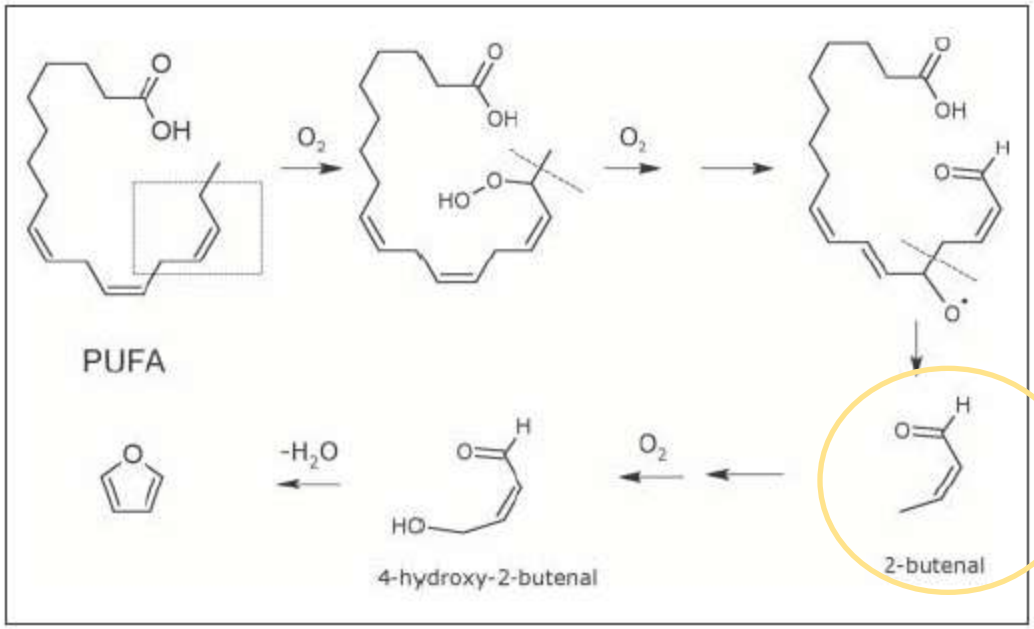

Fig. 2 Proposed lipid oxidation pathway of formation of furan [based on Perez and Yaylayan (2004)].

Perez and Yaylayan, J. Agric. Food Chem. 2004, 52, 6830-6836.

*Owczarek-Fendor et al., J. Agric. Food Chem. 2011, 59, 2368–2376.

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Amino acids as precursors of furan

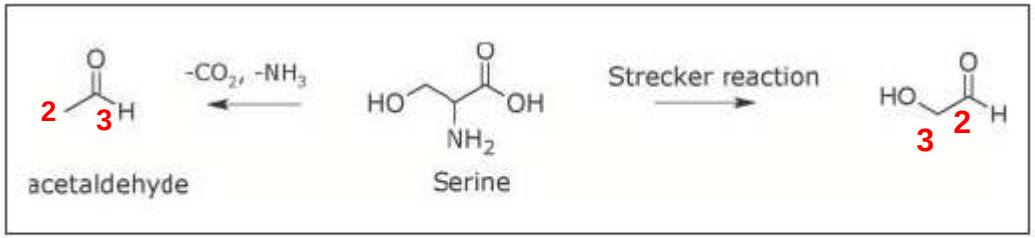
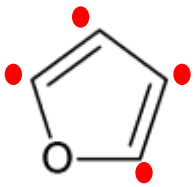


Fig. 3 Thermal decomposition of serine to produce the building blocks of furan [based on Perez and Yaylayan (2004)].

Table 2. Percent Label Distribution in the Parent Furan Generated from Labeled L-Serine

model	M	M + 1	M + 2	M + 3	M + 4
serine	100	0	0	0	0
[1-^{13}C]serine	100	0	0	0	0
[2-^{13}C]serine	0	0	100	0	0
[3-^{13}C]serine	0	0	100	0	0



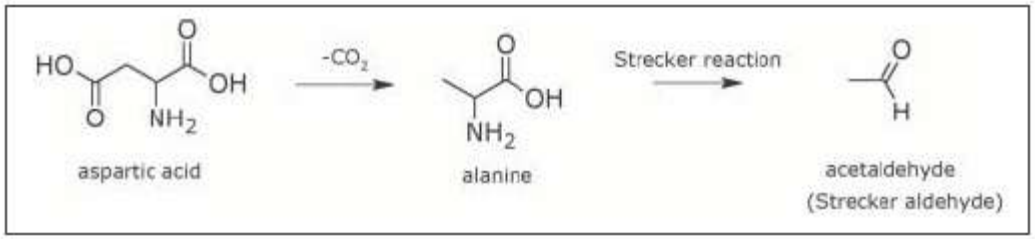


Fig. 4 Thermal decomposition of aspartic acid and alanine to produce one of the precursors (acetaldehyde) of furan [based on Perez and Yaylayan (2004)].

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Sugars as precursors of furan

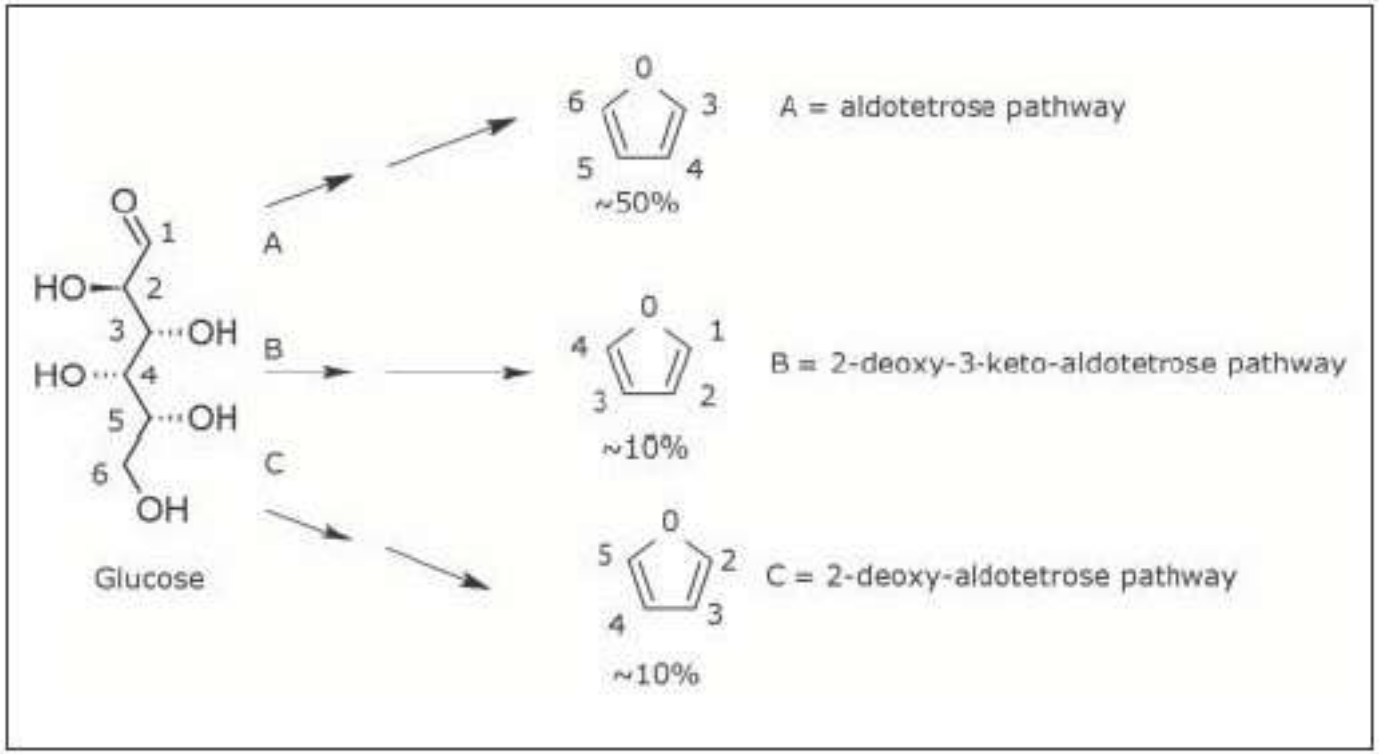

Fig. 5 Origin of carbon atoms incorporated into the furan ring from D-glucose, in D-glucose/serine model system where 30% of furan originated from serine [based on Perez and Yaylayan (2004)].

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Mitigation strategies

Although there is evidence that furan levels can be reduced in some foods through volatilization during cooking (e.g. warming and stirring canned or jarred foods in an open saucepan), there is currently a lack of mitigation strategies that can be employed due to multiple pathways of generation.

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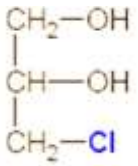
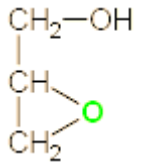
3-MCPD Glycidol





Genotoxic in several *in-vitro* assays

Not genotoxic/mutagenic *in-vivo* (mice & rats)

Tumours in kidney, testes, mammary gland (F344 / SD rats)

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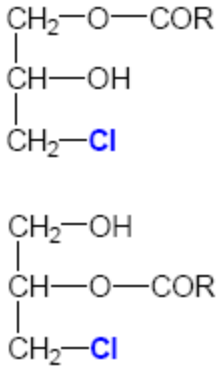
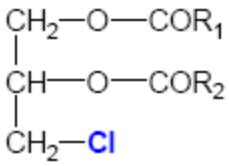
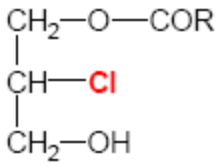
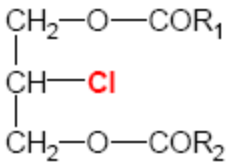
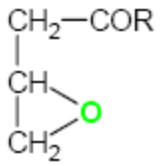
Glycidol and 3-mcpd esters in refined oils











- fatty acids, n=7 (C12:0, C14:0, C16:0, C18:0, C18:1, C18:2, C18:3)
- number of individual esters?

14 **49** **7** **28** **7**

Σ 63 **Σ 35**

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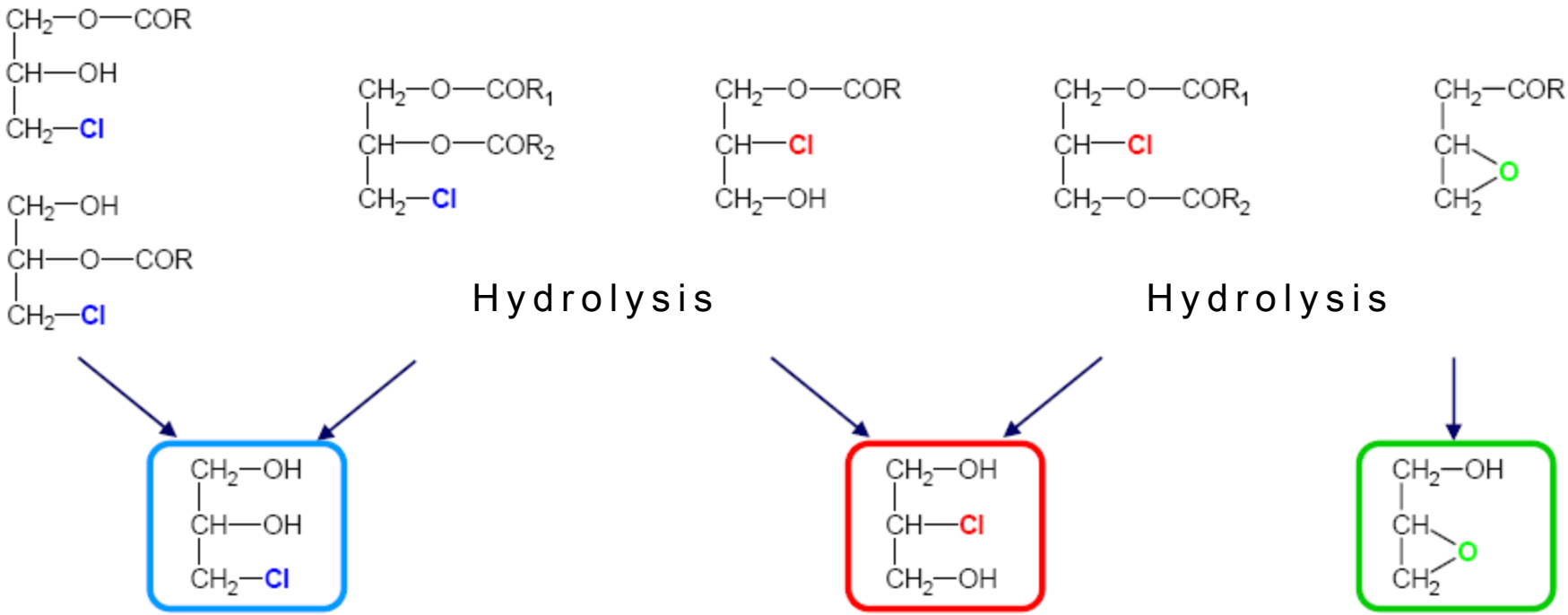
Analytical approaches: Indirect method



- individual esters converted to a single substance: **3-MCPD**, **2-MCPD** or **glycidol** (these are quantified and expressed as a sum “bound form“)

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Summary of reported amounts of 3-mcpd esters in various oils

Oils and Fats	Range (mg/kg)
Unrefined vegetable oils	<0.1 to 0.3
Refined vegetable oils	<0.15 - 18.8
Refined palm oil	1.1 - 10.0
Palm fractions	0.4 - 1.4
Refined coconut oil	1.4 - 1.8
Refined hazelnut/walnut oil	1.2 - 19.0
Refined olive oil	<0.3 - 2.5
Refined salmon oil	0.7 - 13.0
Margarine (fat portion)	<0.15 - 7.7
Coffee creamer	<0.1 - 0.4

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Summary of reported amounts of Glycidol esters in various oils

Food	Range (mg/kg)
Unrefined vegetable oils	<0.10
Refined vegetable oils	<0.1 - 4.1
Cooking oil	<0.1 - 28
Refined palm oil	0.3 - 10
Palm shortening/olein	0.4 - 15.6
Refined hazelnut/walnut oil	0.5 - 1.4
Margarine (fat portion)	<0.15 - 5.0
Mayonnaise (fat portion)	<0.15 - 0.3
Infant formula (fat portion)	<0.15 - 3.0

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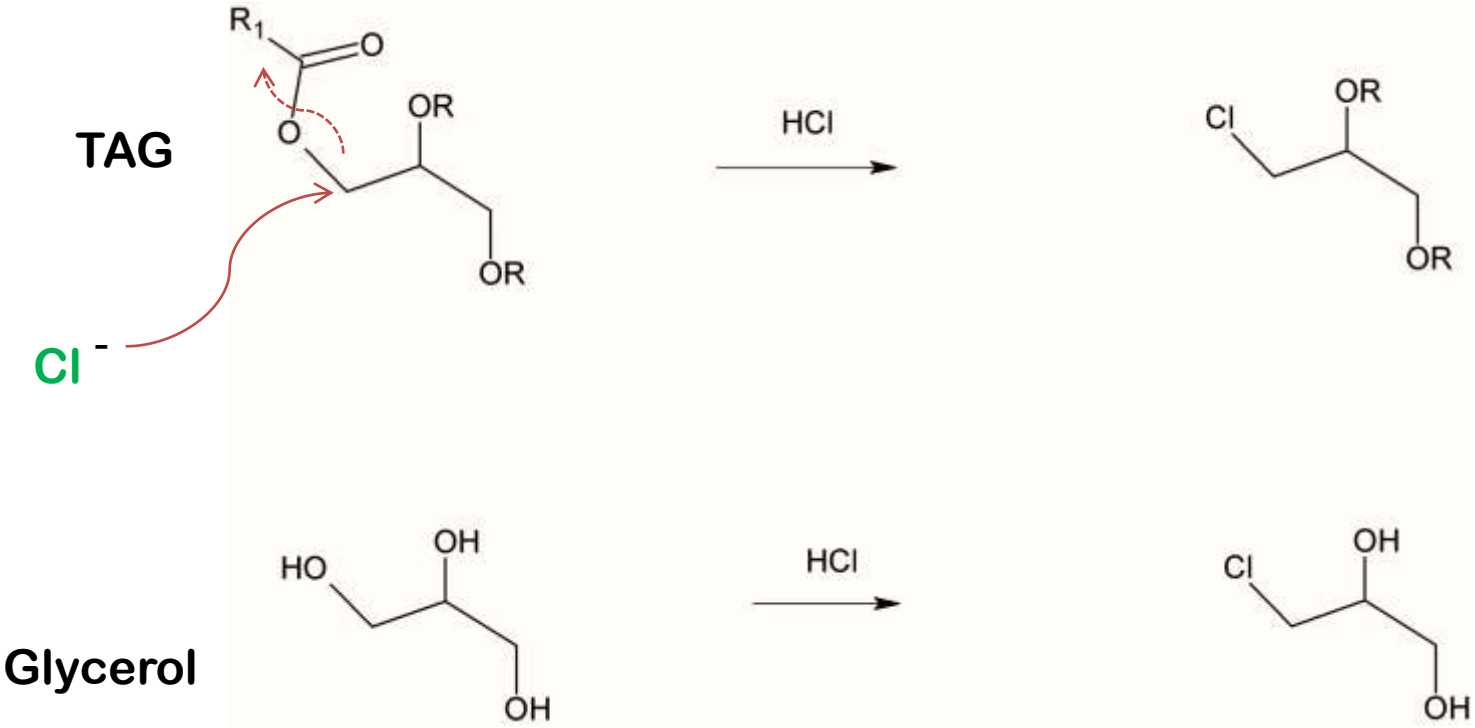
How 3-mcpd esters are formed in refined oils?



Simple substitution?

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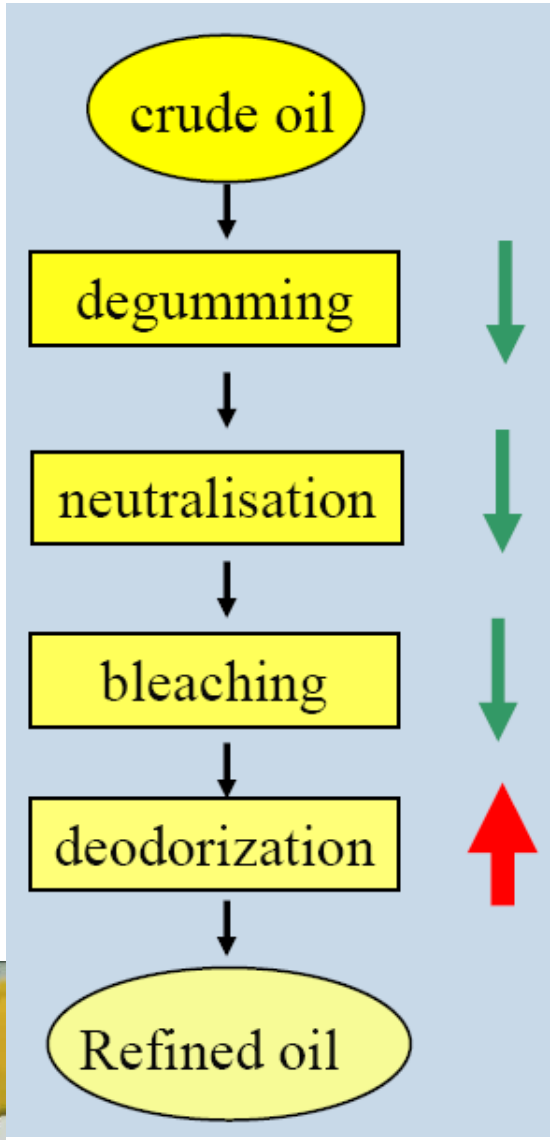
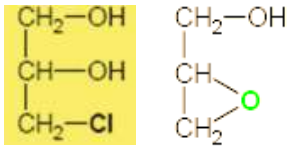
Where the chlorine is coming from?





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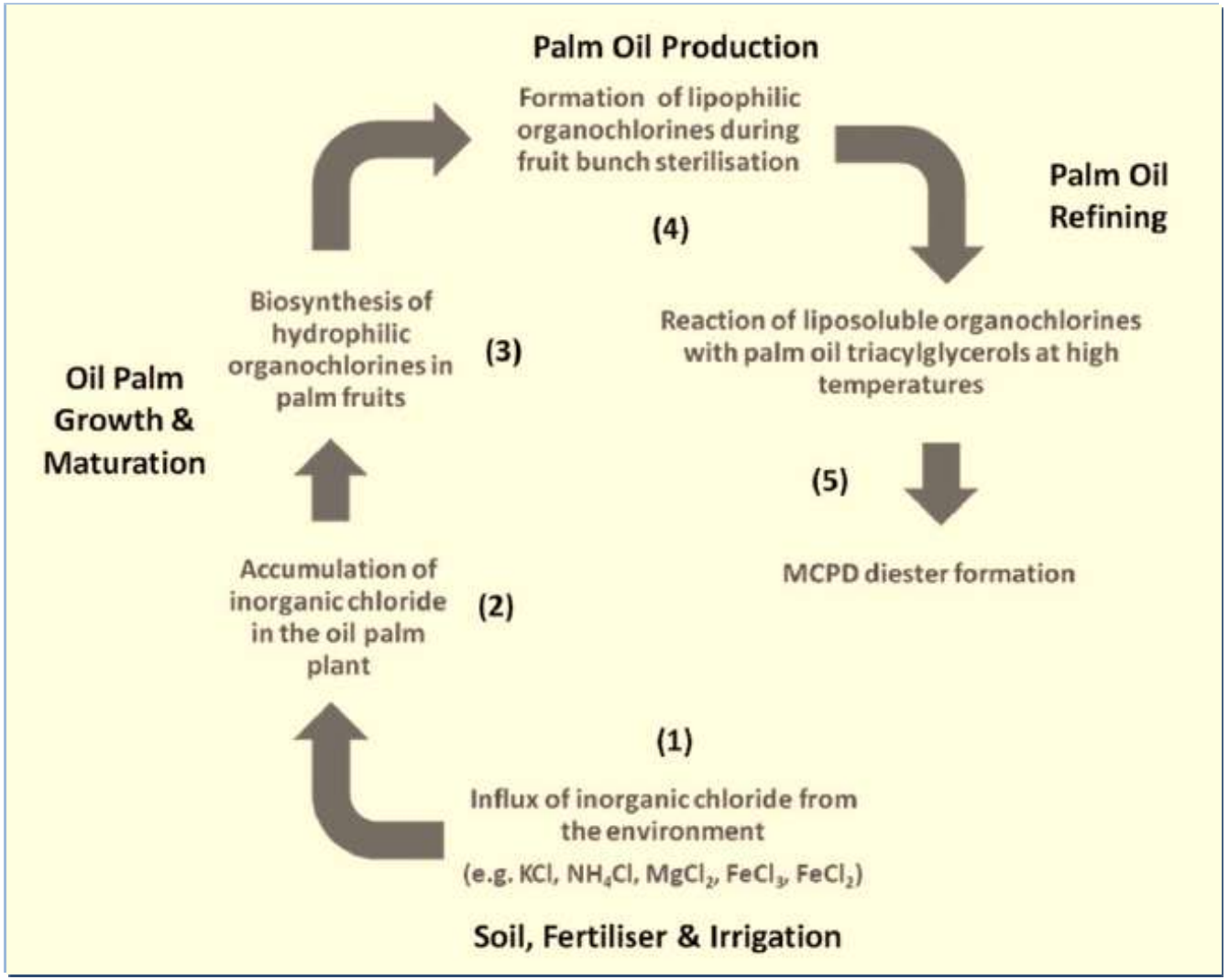

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2011, 1–8, iFirst

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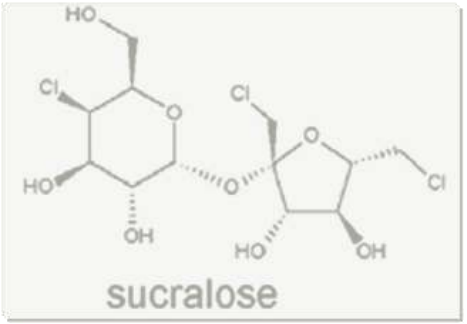
Hydrophilic organo-chlorine compounds



-HCl

+ Glycerol

Chloropropanols

A. Rahn, V.A. Yaylayan / Food Chemistry 118 (2010) 56–61

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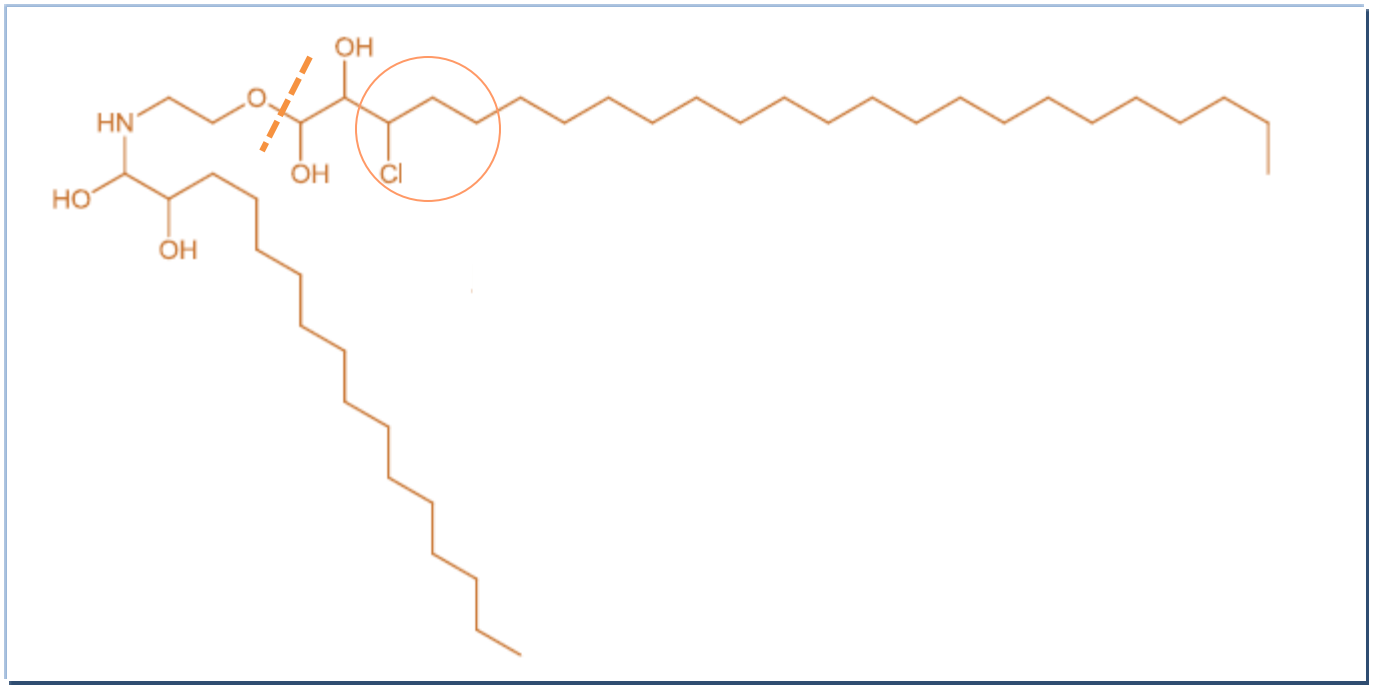
Lipophilic organo-chlorine compounds



-HCl

TAG

3-MCPD esters

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2011, 1–8, iFirst

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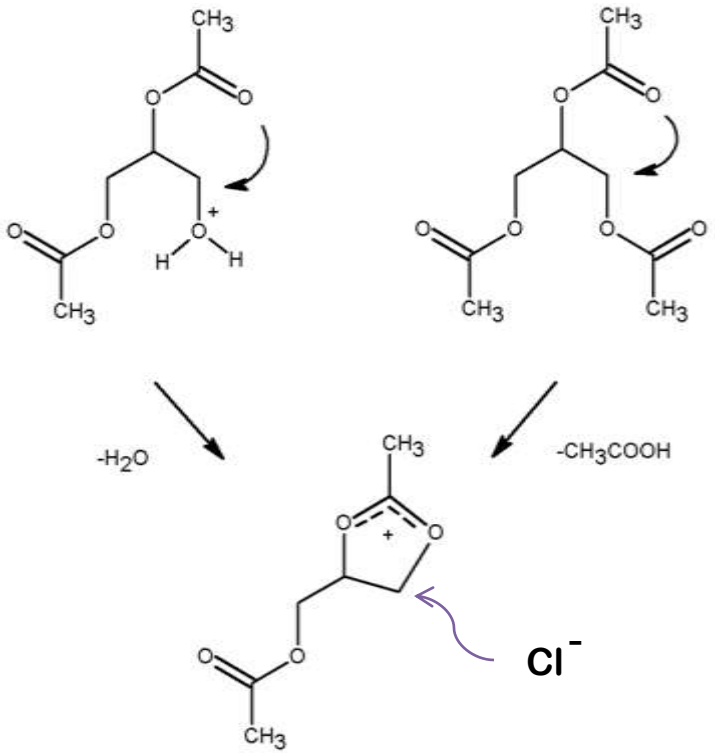
What is the mechanism of chlorination?



Cyclic acyl oxonium ion

Rahn & Yaylayan, Eur. J. Lipid Sci. Technol. 2011, *113*, 330–334

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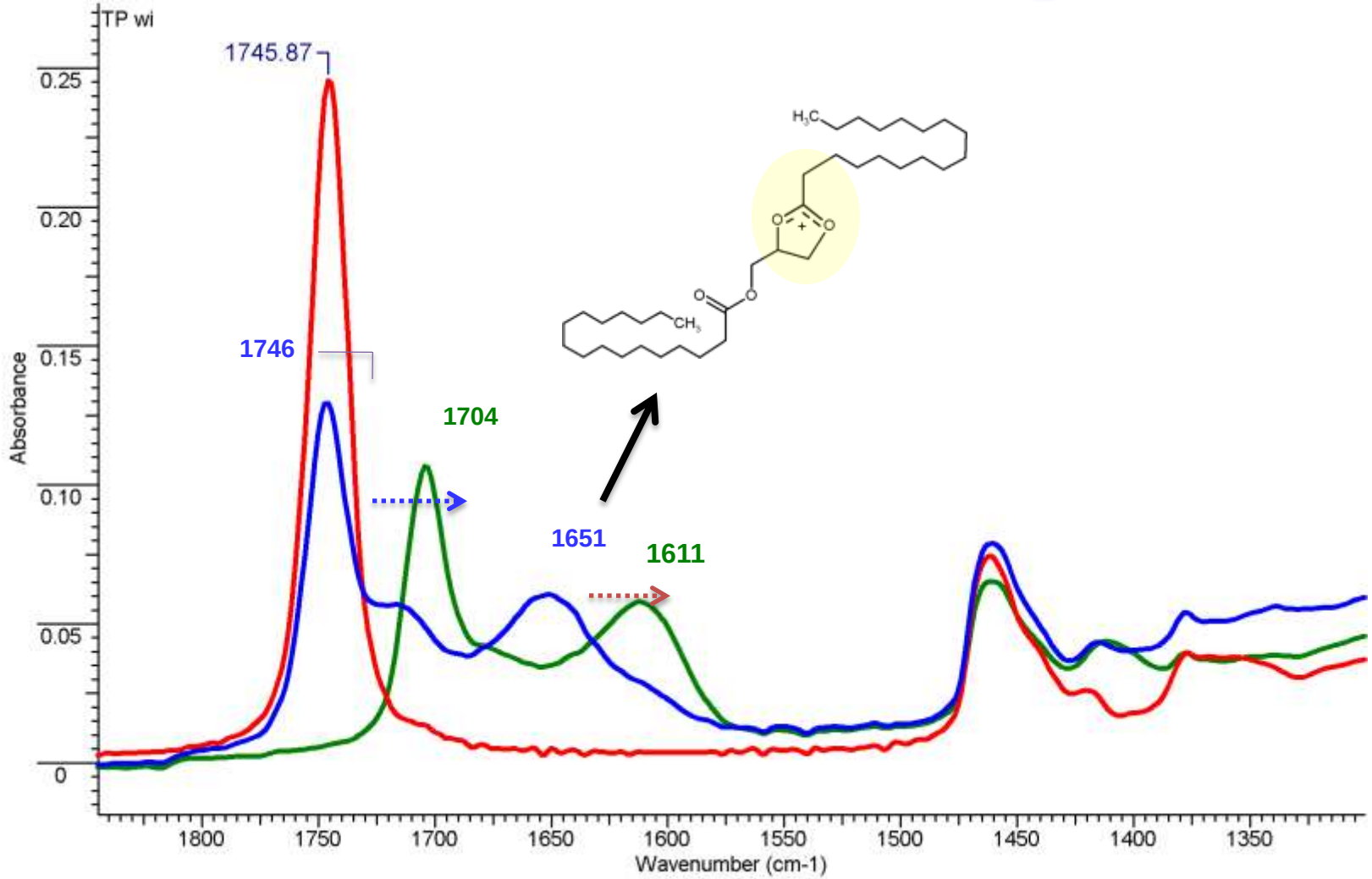
FTIR experiment with $ZnCl_2$ at 90°C



Rahn & Yaylayan, Eur. J. Lipid Sci. Technol. 2011, *113*, 330–334

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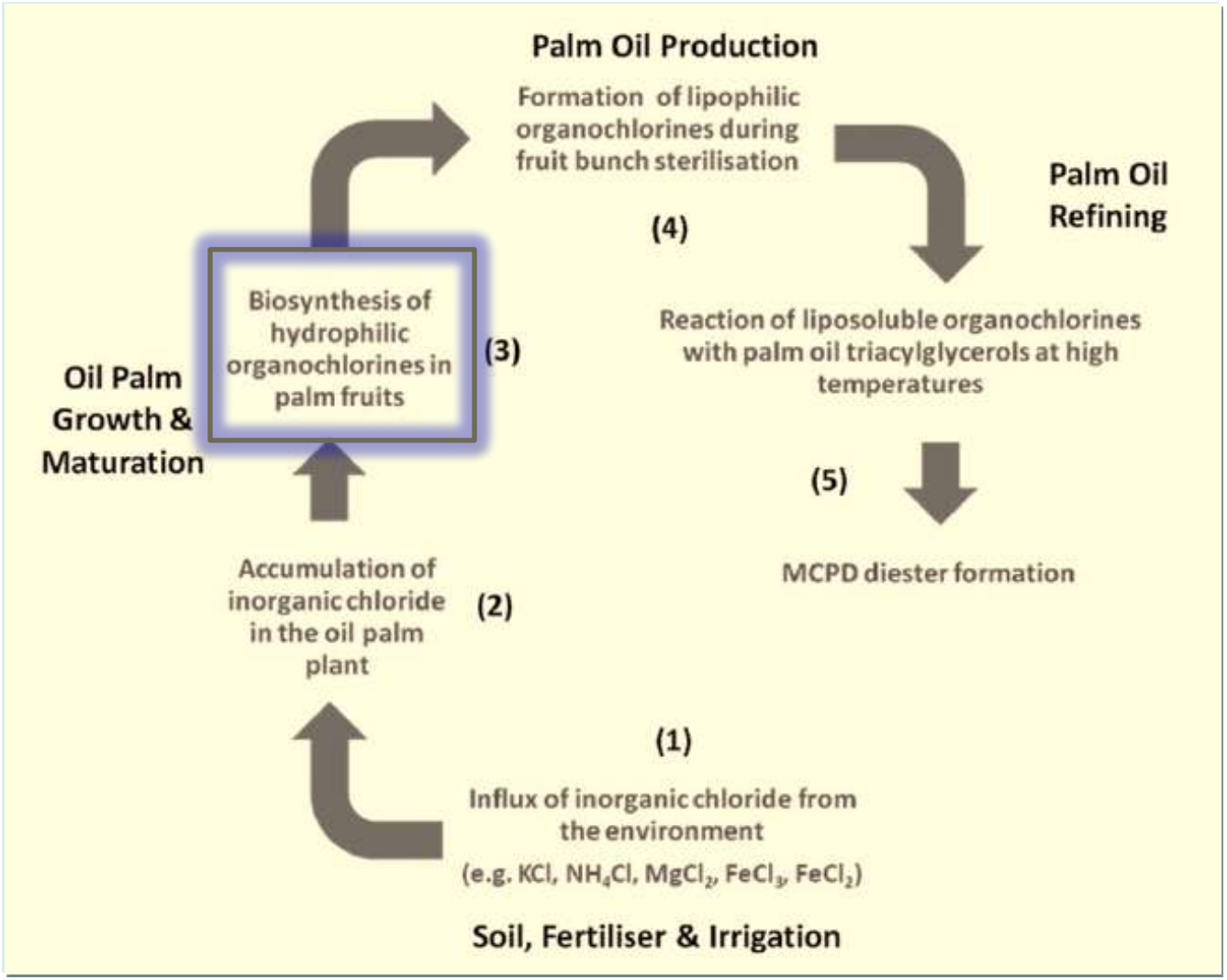
Most efficient mitigation strategy is the removal of chlorine precursors in stage 3



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CONCLUSION

An emerging food safety issue?

Knowledge of the precursors and in-depth understanding of the mechanism of formation of thermally generated toxicants are critical to propose mitigation strategies.

Isotope labeling is an indispensable tool to identify precursors and formation pathways.

Interdisciplinary efforts are needed from all stakeholders to effectively address the research needs of food chemical toxicants

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THANK YOU

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