Report No. C032

Date: October 2014

The chemistry, formation and occurrence of 3-aminopropionamide (3-APA) in foods: a review prepared for the UK Food Standards Agency

Colin G. Hamlet[1]*

Lisa Asuncion[1]

[1] Premier Analytical Services, Lord Rank Centre, Lincoln Road, High Wycombe, Bucks HP12 3QR, UK

*Phone: +44 (0)1494 428211

*Fax: +44 (0)1404 428114

*email: colin.g.hamlet@premierfoods.co.uk

First draft: June 2014

Final version: October 2014

1. Summary

3-aminopropionamide (3-APA), previously identified as a transient intermediate in the formation of AA, should be regarded as an efficient precursor of acrylamide (AA). In fact, far from being a transient intermediate, 3-APA has been shown to accumulate in foods where it can be formed from asparagine by mechanisms involving both thermal and biochemical (enzyme) pathways. This review brings together recent information from the peer reviewed scientific literature and considers the formation, occurrence and toxicology of 3-APA in the context of its relationship to AA. Methods of analysis for 3-APA are discussed together with recommendations for additional work.

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3. Abbreviations

3.1 General abbreviations

AA	acrylamide
3-APA	3-aminopropionamide, 3-aminopropanamide, beta-alanine amide
APCI	atmospheric pressure chemical ionisation
Asn	asparagine
HPLC-FLD	combined high performance liquid chromatography with fluorescence detector
ESI	electrospray ionisation
LC-MS/MS	combined liquid chromatography with mass spectrometry
TTC	threshold of toxicological concern

4. Introduction

3-aminopropionamide (3-APA) is an industrial chemical that does not appear to have any direct commercial applications although it is widely available as a small molecule starting material / intermediate for general chemical syntheses. Interestingly, the 3-APA moiety, incorporated within various carrier molecules, has found use in antitumor applications such as clinical research into the treatment of non-small cell lung cancer. A 3-APA moiety, linked to a carrier molecule (e.g. 4-anilinoquinazoline), was used to deliver an acrylamide derivative via a retro Michael reaction (of 3-APA) into the intracellular environment (Galvani et al 2013): The acrylamide derivative then reacted with the thiol groups of an epidermal growth factor which was believed to be responsible for tumour cell proliferation (Carmi et al 2012).

In early 2000 Zyzac et al (2003) identified 3-aminopropionamide (see Figure 1) as a transient intermediate in model system studies of acrylamide (AA) formation from reactions of asparagine (Asn) initiated by reducing carbohydrates or aldehydes. However, as only a slight increase in the yield of acrylamide was observed when compared to Asn, the role of 3-APA was not considered further.

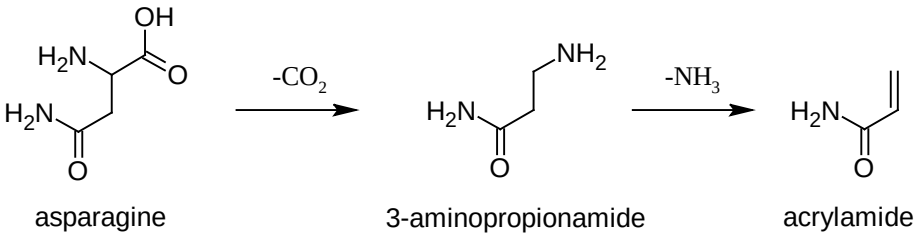

Figure 1. Chemical structure of 3-aminopropionamide and relationship to asparagine and acrylamide

3-APA can be considered as the biogenic amine of Asn, and subsequent studies shown it may be formed during the storage of foods by an enzymatic decarboxylation of Asn. Furthermore it would now seem that 3-APA can generate acrylamide in relatively high yields (Granvogl et al 2004), when compared to Asn, particularly when heated under certain reaction conditions. Hence the presence of 3-APA in foods, that are also subjected to thermal processing, might be considered as an additional source of AA.

This review brings together recent information concerning the occurrence of 3-APA in foods, mechanism(s) of formation, the toxicological potential of 3-APA and methods of analysis. It is anticipated that this review will provide the justification for further investigations of foods and the effects of processing on 3-APA / acrylamide formation.

5. Chemistry, formation and occurrence

5.1 Physical and chemical properties.

The chemical and physical properties of 3-APA are given in Table 1 and Table 2.

Table 1. Chemical summary[a,b] for 3-APA

Parameter	Value
Average mass	88.108398 Da
CASRN	4726-85-6
GCID	256812
H-Bond Acceptor	2
H-Bond Donor	2
Molecular formula	$C_3H_8N_2O$
Rotatable Bond Count	2
SMILES	C(CN)C(=O)N
Synonyms	3-aminopropanamide 3-aminopropionamide beta-alanine amide propanamide, 3-amino
Tautomer Count	3

[a] Aggregated Computational Toxicology Resource (ACToR), http://actor.epa.gov/actor/faces/ACToRHome.jsp (accessed 01/07/2014); [b] PubChem Compound search, http://pubchem.ncbi.nlm.nih.gov/# (accessed 01/07/2014)

Table 2. Predicted physical properties of 3-APA[a]

Parameter	Value
Average mass	88.108398 Da
Boiling point (°C)	306.5 (760 mm HG)
Density	1.1±0.1 g/cm^3
Flash point	139.2±23.2 °C
LogP	-1.81±0.26
Refractive index	1.47

[a] CSID:167316, http://www.chemspider.com/Chemical-Structure.167316.html (accessed 01/07/2014)

5.2 Formation mechanisms

5.2.1 Thermal formation

Shortly after the discovery of AA in processed foods (Tareke et al 2002) several studies identified that the amino acid Asn provides the backbone of AA formed in thermally processed foods. While simple decarboxylation / deamination reactions might yield AA from Asn (see Figure 1), the detailed formation pathway from Asn is complex and still under investigation. A general reaction scheme for the formation of AA from Asn is given in Figure 2. The mechanism proceeds with the formation of a

Schiff base from the reaction of a reactive carbonyl (e.g. glucose) with Asn followed by decarboxylation to give an unstable intermediate, i.e. and azomethine ylide (Mottram et al 2002; Stadler et al 2002; Yaylayan et al 2003; Zyzac et al 2003). Stadler et al (*2004*) reported that the sugar-Asn adduct (Schiff base) was a direct precursor of AA whereas a Strecker type degradation of Asn (Mottram et al 2002) via 3-oxopropanamide, or the corresponding alcohol 3-hydroxypropanamide, was more or less excluded (Stadler et al *2004*). Hydrolysis of the decarboxylated Schiff base gives 3-APA which upon elimination of ammonia yields AA (Yaylayan and Stadler 2005; Zyzac et al 2003). The alternative pathway to AA from the decarboxylated Schiff requires the elimination of an imine.

The Maillard reaction can produce many reactive carbonyls which may form Schiff bases with Asn, and the effectiveness of these different carbohydrate moieties on AA formation has been investigated by a number of research groups. Some of the carbonyls relevant to 3-APA / AA formation include reducing carbohydrates (Stadler et al 2002), hydroxycarbonyls, dicarbonyls (Mottram et al 2002; Amrein et al 2004; Stadler et al 2004; Schieberle et al 2005), 2-oxo acids (Schieberle et al 2005), 2-oxo aldehydes (*ibid*) or simply aldehydes (Zyzac et al 2003), alkadienals and lipid oxidation products (Hidalgo et al 2009, 2010; Zamora and Hidalgo 2008) and 5-hydroxy-2-furfural (HMF) (Gokmen et al 2012). For example, HMF, one of the main hexose dehydration products (Maillard), was shown to be a very potent generator of both AA and 3-APA from Asn compared to saccharides such as glucose (*ibid*). This effect was attributed, in part, to the relatively low melting point of HMF and hence more thermodynamically favourable formation of amine condensation products during heating. Since sugar dehydration and Maillard reactions occur simultaneously in thermally processed foods, the contribution of HMF and other reactive carbonyls (e.g. from sugar dehydration) should be considered in the AA risk assessment. Foods that contain relatively high amounts of HMF include dark roasts coffees and malt.

The finer detail of the mechanism of AA formation is evidently complex and still under discussion. The relative amounts of AA and 3-APA formed may depend not only in the nature of the carbonyl compound that reacts with Asn but also on conditions of temperature, time, water content, pH and the physical state of the reactants.

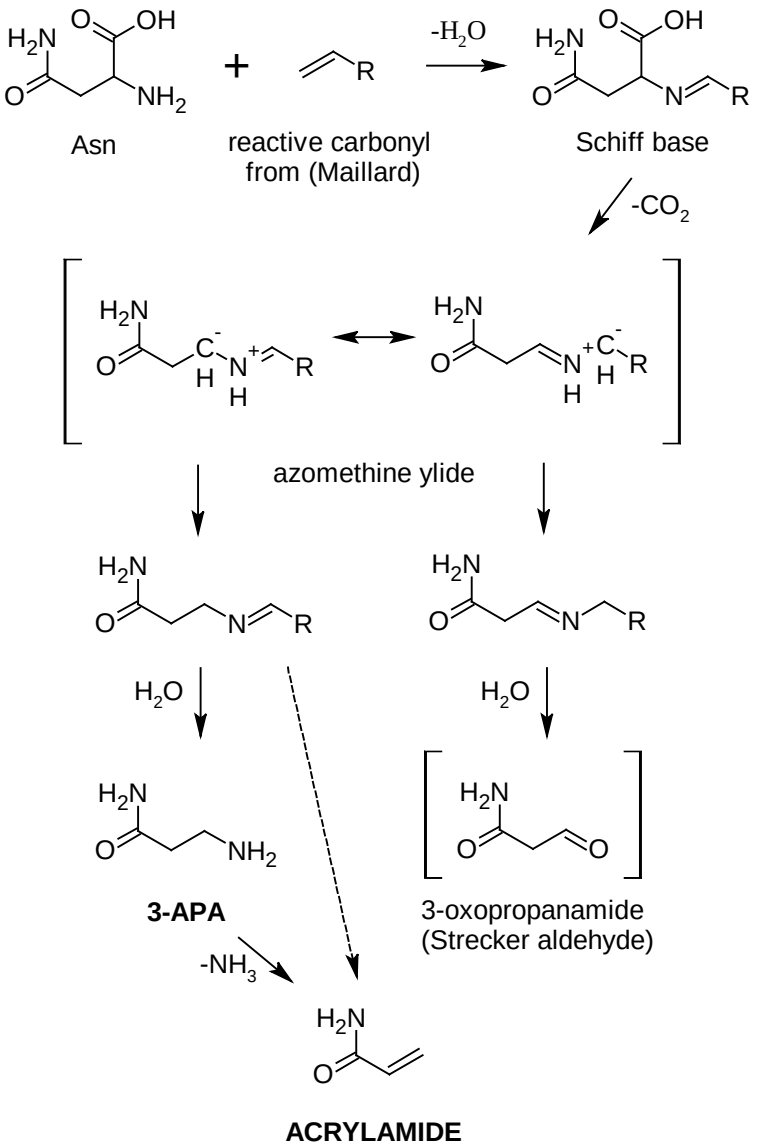

Figure 2. Proposed mechanism for the thermal formation of 3-APA and AA (Stadler et al 2004; Yayalan et al 2005)

Studies of the thermal degradation of 3-APA to AA under aqueous or low water conditions at temperatures between 100°C and 180°C have demonstrated that it is a very effective precursor of AA in heated foods (Granvogl et al 2004; Schieberle et al 2005). For example, Granvogl et al (2004) found that heating of 3-APA in aqueous model systems always generated more AA than in the same reaction using Asn: the highest yields were circa 28 mol% in the presence of carbohydrates (170°C aqueous buffer) and about 63 mol% in the absence of carbohydrates under the same conditions. Heating of propanoic acid amides with an amino or hydroxyl group in the α-position, e.g. 2-aminopropionamide,

were ineffective in generating AA indicating that elimination of the amino group occurred only at the β-position (*ibid*).

Zamora et al (2009) studied the deamination reaction in model systems of 3-APA and 3-(alkylamino)propionamides (benzyl, phenylethyl, butyl, and octyl). The formation of AA occurred at almost neutral or basic pH values and yields appeared to depend not only the type of aminopropionamide involved but also on the water activity. The occurrence of 3-(alkylamino)propionamides in foods is not known. On the other hand, the role of carbonyl compounds in the AA produced, appeared to have less impact than either the type of amine or the water activity.

Cai et al (2014) found that chlorogenic acid, present at high concentrations in some raw foods, could influence AA formation. Factors that appeared to favour increased AA formation from Asn / glucose model systems in the presence of chlorogenic acid included: increased formation of HMF, which acts as a more efficient precursor than glucose to form acrylamide; decreased activation energy for conversion of 3-APA to AA (from 173.2 to 136.6kJ/mol), and hence increased rate of deamination; a high redox potential during the Maillard reaction which may prevent the destruction of AA by free radicals.

5.2.2 Enzymatic formation

In addition to the non-enzymatic (Maillard) decarboxylation reactions of Asn, Granvogl et al (2004) proposed a biochemical pathway to 3-APA involving decarboxylases present in raw materials (see Figure 3).

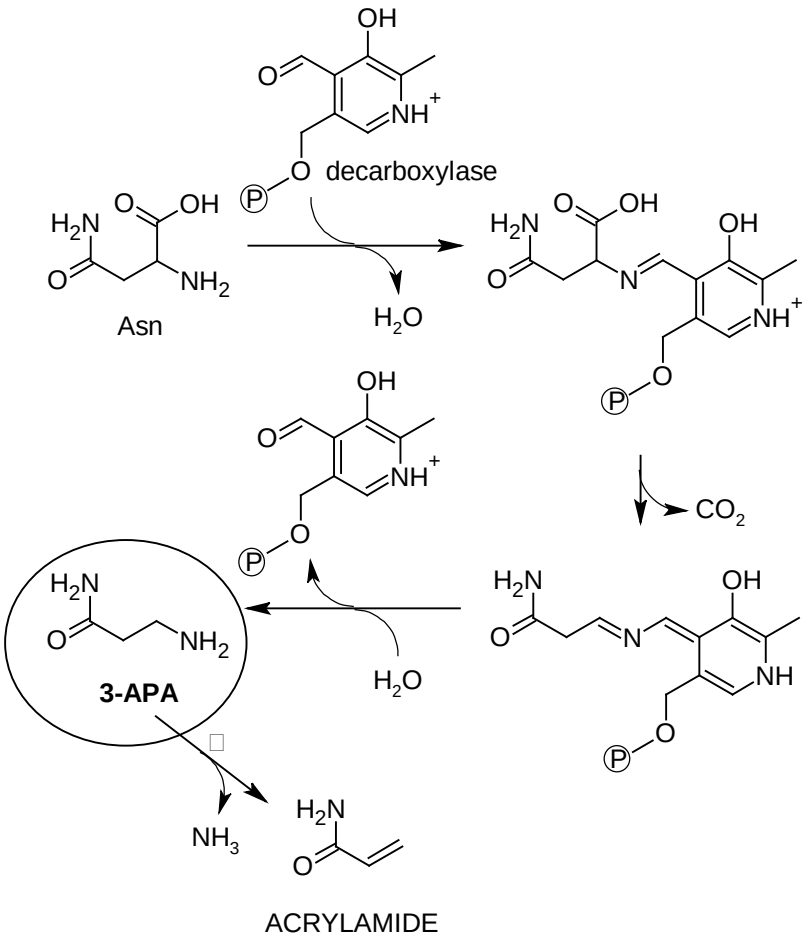

Figure 3. Biochemical pathway for the conversion of Asn into 3-APA (adapted from Granvogl et al 2004)

Using a newly developed method of analysis based on LC-MS/MS, Granvogl et al (2004) were able to show that 3-APA was present in different potato cultivars and that amounts increased during storage or after crushing of the cellular material. While it was not known if Asn decraboxylases were present in potatoes, the authors were able to demonstrate the generation of 3-APA from Asn in model reactions with a variety of commercially available decarboxylases. Among the non-specific decarboxylases selected, histidine decarboxylase was the most effective although only circa 0.1% of Asn was converted to 3-APA, indicating that this pathway was a minor process.

5.3 Foods

Although published data is still rather limited, 3-APA would appear to be widespread in raw and processed cereal, cheese, cocoa and potato products (Table 3).

Moderate amounts of 3-APA (34-289 µg/kg) have been reported in corn flakes, rice-cocoa based snack, rusk, wheat snack and a wheat bread snack. A higher amount of 3-APA was found in Pop corn (1878 µg/kg). No detailed description was available for this product and it is not known if this could be attributed to additional ingredients, e.g. sugar, or the corn raw material.

Table 3. Amounts of 3-APA measured in food products

Commodity	number samples	3-APA (µg/kg)		Reference
		mean	range	
Breakfast cereal, corn flakes	1	-	171	Granvogl and Schieberle (2007)
Chocolate	1	-	~3000	Granvogl and Schieberle (2007)
Cocoa beans				Granvogl and Schieberle (2007)
unfermented	2	214	115-313	
7 days fermented	2	473	155-792	
unfermented, roasted	2	1788	1498-2078	
7 days fermented, roasted	2	2647	1818-3476	
Cocoa mass, commercial	3	1689	217-3014	Granvogl and Schieberle (2007)
Coffee				Granvogl and Schieberle (2007)
ground	1	-	122	
extract	1	-	241	
Coffee				Bagdonaite et al (2006)
green	3	<17	-	
roasted	2	<17	-	
Coffee surrogate	1	-	453	Granvogl and Schieberle (2007)
Gouda Cheese				Granvogl and Schieberle (2006)
uncooked	1	-	4	
cooked[a]	1	-	1324	
Popcorn	1	-	1878	Granvogl and Schieberle (2007)
Potatoes, ware				
cool stored[b]	3	11500	9500-14000	Bagdonaite et al (2006)
fresh	5	215	136-294	Granvogl et al (2004)
cool stored[C]	5	365	188-748	Granvogl et al (2004)
warm stored[d]	5	787	323-1554	Granvogl et al (2004)
Rice-cocoa snack	1	-	199	Granvogl and Schieberle (2007)
Rusk	1	-	34	Granvogl and Schieberle (2007)
Wheat snack	1	-	288	Granvogl and Schieberle (2007)
Wheat bread snack	1	-	289	Granvogl and Schieberle (2007)

[a] 30 min at 180°C; [b] 20 weeks @ 4-8°C; [C] 5 weeks @ 20°C; [d] 12 days @ 35°C

5.3.1 Thermal processing

Although a sample of fresh cheese contained a low amount of 3-APA (4 µg/kg) its concentration increased to ~1300 µg/kg on heating (Granvogl and Schieberle 2006).

Granvogl and Shchieberle (2007) measured 3-APA concentrations in a ground coffee (122 µg/kg) and a coffee extract (241 µg/kg). The authors also reported corresponding amounts of AA of 807 and 1492 µg/kg respectively. However, Bagdonaite et al (2007) did not detect 3-APA in either of two dry processed coffees that had been roasted in an oven at temperatures between 150-240°C over 5-15 min, or in green coffees.

A relatively high concentration of 3-APA measured in commercial cocoa mass was subsequently found to account for amounts found in chocolate (Granvogl and Schieberle 2007). Investigations of unroasted and roasted cocoa beans also showed that thermal generation of 3-APA was the dominant

route. Interestingly, 3-APA concentrations in both fermented unroasted and fermented roasted cocoa beans were significantly higher than in the unfermented products indicating that some biochemical formation of 3-APA had occurred as well as conditions favouring additional formation during heating. The authors also reported the corresponding data for AA and Asn in the cocoa beans studied (see Table 4): concentrations of Asn decreased during fermentation and roasting consistent with the formation of 3-APA / AA according to the mechanisms outlined in 5.2. Amounts of AA however were much increased in the fermented roasted beans compared to the unfermented roasted samples. The authors suggested that this may be due to the lower pH in the beans from the formation of acetic acid during fermentation, which would favour the deamination of 3-APA into AA. No explanation was provided for the relatively low, but not insignificant, amounts of AA measured in the unroasted beans. In contrast to the coffee samples (above), the concentrations of AA were always lower than that of 3-APA in the cocoa beans.

Table 4. Influence of fermentation and roasting on 3-APA, AA and Asn in cocoa beans[a]

Sample	Number of samples	3-APA[b,c] (µg/kg)	AA[b,c] (µg/kg)	Asn[b,c] (mg/kg)
unfermented	2	214	62	611
7 days fermented	2	473	72	557
unfermented, roasted	2	1788	172	512
7 days fermented, roasted	2	2647	630	404

[a] Adapted from Granvogl and Schieberle (2007); [b] Data are mean of cocoa bean samples from Ghana and Sulawesi; [c] Dry weight basis

To further understand the effects of thermal processing on amounts of 3-APA and hence AA formed, Granvogl et al (2007) prepared a range commercial wheat flours and flour produced from wheat grown under different conditions of sulphur fertilization and subjected these to heat treatment. In agreement with the high amount of Asn present in the sulphur depleted flours[1], Table 5 shows that heating led to the generation of very high amounts of acrylamide (1.7-3.1 mg/kg) and 3-APA (40-76 mg/kg). Amounts of 3-APA and AA correlated well with Asn (R^2 0.98 each) and S fertilization (R^2 0.91 and 0.92 respectively) across the seven flours investigated thereby providing additional data in support of the mechanisms given in 5.2.1.

[1] Sulfur (S) fertilization has been long-known to influence the amounts of total free amino acids in plants (Muttucumaru et al 2006).

Table 5. Concentrations of 3-APA and AA generated in experimental and commercial flours after heat treatment

	heat treated (20 min @ 170°C)		non heat treated	
Flour[a]	3-APA (mg/kg)	AA (mg/kg)	Asn (mg/kg)[b]	S (mg/kg)[b]
1	76.01	3.124	5688	660
2	39.58	1.703	3920	840
3	4.84	0.460	426	1280
4	1.42	0.155	142	1430
5	0.38	0.094	35	1580
C1	1.47	0.157	112	1350
C2	1.74	0.213	130	1420

[a] Flours 1-5 grown with varying amounts of S fertilization, flours C1 and C2 were commercial samples; [b] data from non-heat treated flour for correlation.

5.3.2 Effects of storage on potatoes

Granvogl et al (2004) measured 3-APA in five different raw potato cultivars (data given in Table 3, mean 215μg/kg) and showed that amounts could increase on storage (by a factor of ~4) or after crushing the cellular material (by a factor of ~2, 20°C 480 min). The Asn contents of the fresh cultivars ranged from 4.48-6.30 g/kg (mean = 5.09 g/kg). Bagdonite et al (2007) later confirmed the presence of 3-APA in cool stored potato cultivars. Amounts measured were more than a factor of x10 higher that the values reported by Granvogl (2004) although the storage times were significantly longer (~20 weeks v 5 weeks). The influence of storage conditions on amounts of AA generated during thermal processing, previously attributed to changes in potato reducing sugar levels (Foot et al 2007), may require further evaluation with respect to 3-APA.

5.4 Conclusions from chemistry, formation and occurrence

- 3-APA is generated from the thermal decarboxylation / decomposition reactions of a Schiff base formed from a Maillard-type reaction involving Asn and a reactive carbonyl compound
- 3-APA can form AA directly by heating
    - it has been shown to be a more potent precursor of AA than Asn
- Chlorogenic acid can promote the formation of AA from 3-APA in model systems
    - significant amounts of chlorogenic acid are found in green coffee and prunes
- Foodstuffs found to contain significant amounts of 3-APA include: chocolate, raw potatoes, roasted cocoa beans
- Thermal processing can increase the generation of 3-APA e.g. cheese, cocoa beans, cocoa mass, popcorn

    - Fermentation of cocoa beans would appear to produce more AA during roasting
- Amounts of 3-APA in ware potatoes would appear to increase significantly with increasing storage time / temperature.
    - the impact of such increases on AA formation is not known

6. Toxicological aspects

Toxicological studies of 3-APA have not been undertaken and hence expert evaluations are not available. However, the use of (Quantitative) Structure–Activity Relationship ((Q)SAR) models, such as ToxTree, can enable structure-based predictions to be made for a number of toxicological endpoints and mechanisms of chemical action where compound specific toxicity data is lacking.

A summary of the key toxicity predictions from the application of the (Q)SAR program Toxtree[2] (Benigni et al 2011) are given in Table 6. While there were no positive alerts for invitro mutagenicity (Ames test by S. Tryphimurium), Toxtree predicted at least one positive structural alert for invivo mutagenicity (micronucleus assay). Predictions of carcinogenicity (genotoxic and non-genotoxic) and mutagenicity rulebase by ISS were negative.

[2] Toxtree version 2.6.6. Available at: http://toxtree.sourceforge.net [Accessed 02.10.14]

Table 6. Summary of toxicity predictions[a] for 3-APA

Decision Tree	Toxic Hazard
Cramer rules	High (Cramer class III)
Cytochrome P450-mediated metabolism	N-dealkylation: $NH_2COCH_2CHO + NH_3$ Amine hydroxylation: $NH_2COCH_2CH_2NHOH$ N-oxidation: $NH_2OCOCH_2CH_2NH_2$
DNA binding alerts	Schiff base formation
Invitro mutagenicity (Ames test by S. Tryphimurium)	Negative
Carcinogenicity (genotoxic and non-genotoxic) and mutagenicity rulebase by ISS[3]	Negative for genotoxic and non-genotoxic carcinogenitity
Kroes[b] structure based threshold of toxicological concern (intake dependent assessment)	Intake ≤90 µg/day: Substance would not be expected to be a safety concern Intake >90 µg/day: Risk assessment requires compound specific toxicity
Structure alerts for the in vivo micronucleus assay in rodents[c]	At least one positive structural alert for the micronucleus assay (Class I)
Protein binding alerts	Schiff base formation
Structural alerts for functional group identification	At least one functional group alert (Class I)

[a] Benigni et al 2011; [b] Kroes et al 2004; [c] Benigni et al 2009

Since 3-APA may be an effective precursor of acrylamide under certain reaction conditions, its presence in foods prior to thermal processing should be considered a risk factor (acrylamide).

6.1 Conclusions from Toxicological aspects

- Toxicological evaluations of 3-APA have not been preformed
- Application of the (Q)SAR model Toxtree gave at least one structural alert for in vivo mutagenicity (micronucleus assay in rodents)
    - there were no structural alerts for genotoxic and non-genotoxic carcinogenitity and in vitro mutagenicity

[3] Istituto Superiore di Sanità

7. Methods of analysis

Granvogl et al (2004) and Schieberle et al (2005) were the first to develop a method to confirm and quantify amounts of 3-APA generated in model systems and potato cultivars based on LC-MS/MS of the derivative of 3-APA formed from by reaction with dansyl chloride (see Figure 4).

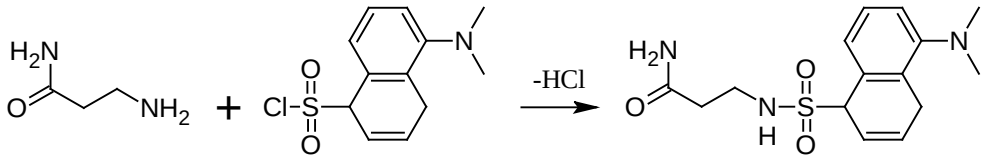

Figure 4. Reaction scheme for the derivatisation of 3-APA with dansyl chloride

For the quantification of 3-APA in potatoes, samples were blended with water and glycinamide internal standard prior to a protein precipitation treatment using Carrez II and II. After centrifugation and pH adjustment, extracts were stirred with dansyl chloride in the dark. The resulting derivatives of 3-APA were extracted with DCM, dried and reconstituted in acetonitrile / formic acid prior to LC-MS/MS. Prepared extracts were separated on a 150 mm x 2.0 mm i.d. Synergy max RP 80 Å column before passing to a positive ion electrospray interface (ESI) of a triple quadrupole mass spectrometer: Separation was attained at a flow rate of 0.2 ml/min and a gradient program was set up with (A) formic acid in water (0.1% W/V) and (B) formic acid in acetonitrile (0.1% w/v); 20% (B) increased to 50% over 15 min. The following precursor to product ion transitions were monitored:

	Precursor ion (*m/z*)	Product ion (*m/z*)	
3-APA	322	170	156
glycinamide	308	170	156

For the measurement of 3-APA in cheese (Granvogl and Schiebberle 2006) the protein precipitation step was omitted and the supernatant was defatted with hexane whereas for cocoa, coffee and cereal products (Granvogl and Schieberle (2007), a defatting step was incorporated into the original procedure of Granvogl et al (2004).

Although no method performance data was reported, the limit of quantification of 3-APA by LC-MS/MS would appear to be in the low µg/kg range.

Bagdonaite also measured 3-APA in coffee and potatoes (Bagdonaite et al 2007) as 3-APA / dansyl chloride derivatives but used HPLC with fluorescence detection (HPLC-FLD). A similar strategy was employed for the preparation of extracts although no carrez protein precipitation or defatting of coffee

samples was employed. The dansyl chloride derivatives were separated by reversed phase HPLC using acetonitrile / water gradient elution with the fluorescence detector set at λ_{Ex} = 320 nm and λ_{Em} = 500 nm. The limits of detection (LOD) and quantification (LOQ) were determined as 17 and 30 µg/l respectively, RSD 3.2%.

Recently, Gökmen et al (2012) followed the formation of 3-APA (and other intermediates) directly by LC/MS during the heating of Asn with glucose. Reaction extracts in 10 mM formic acid were separated on an Atlantis T3 column (Milford, MA, USA) reverse phase column using an isocratic mixture of 10 mM formic acid: methanol (70:30 w/v) as the mobile phase prior to detection using either positive ESI-SIM (*m/z* 89 $[MH]^+$; *m/z* 72 $[MH\text{-}NH_3]^+$) or positive ion atmospheric pressure chemical ionisation (APCI) on a hybrid quadrupole-Orbitrap mass spectrometer (m/z 50 – 300). The authors reported that compared to positive ESI, positive APCI provided better sensitivity for the reaction products such as 3-APA.

7.1 Conclusions from methods of analysis

- 3-APA, isotopically labelled analogues of 3-APA (e.g. $H_2NCD_2CD_2CONH_2$•HCl) and glycinamide are all commercially available (as the hydrochloride salts)
- The formation of a stable sulphonamide derivative of 3-APA (using dansyl chloride) followed by LC-MS/MS detection has been applied successfully to cereal products, cheese, cocoa products, coffee beans and potatoes
- HPLC-FLD of 3-APA dansyl chloride derivatives has also been used for coffee beans and potatoes
- 3-APA has been analysed directly in heated model systems of Asn and glucose using LC/MS
    - positive ion APCI may offer more sensitivity than positive ion ESI
    - the method has not been extended to foods
- None of the methods used to measure 3-APA have been fully validated

8. Future prospects and conclusions

On the basis of recent studies, 3-APA must be considered an important precursor of AA. Not only does 3-APA accumulate in both raw and processed foods but its thermal conversion into AA appears to be more effective than the corresponding generation from Asn. Furthermore, model studies have indicated that processing conditions such as water activity and / or the presence of certain compounds in foods such as HMF or chlorogenic acids may play a role in determining amounts of AA formed from 3-APA during heating.

While researchers have begun to look at the conversion of 3-APA into AA in the laboratory, data on 3-APA in foods is still lacking. To date, chocolate, raw potatoes and roasted cocoa beans would

appear to be significant sources. Thermal processing has also been shown to increase the generation of 3-APA e.g in cheese and popcorn.

While it has been shown that the biogenic generation of 3-APA may be less efficient than the thermal pathway, very high amounts of 3-APA (~12 mg/kg) have been reported to form during the storage of raw potatoes. Furthermore, the contribution of 3-APA to AA generated in ware potatoes (previously attributed to changes in sugar concentrations) has not yet been quantified.

Although an expert toxicological assessment of 3-APA has not yet been undertaken, application of the (Q)SAR model Toxtree (Benigni et al 2011) indicated at least one structural alert for in vivo mutagenicity (micronucleus assay in rodents): no alerts were given for in vitro mutagenicity (Ames test) and carcinogenicity (both genotoxic and nongenotoxic). Since 3-APA has been shown to be a potent precursor of AA, regulators may consider its presence in foods, which might be subject to thermal processing, as an additional source of AA.

Current methods of analysis for 3-APA require the formation of a stable sulfonamide derivate (using dansyl chloride) for detection by LC-MS/MS and appear to be applicable to a wide range of foodstuffs. The direct determination of 3-APA, also using LC-MS/MS, appears attractive and may also afford the simultaneous determination of AA. However, the latter methods have been developed for use with pure reference chemicals and have not yet been developed for more complex matrices.

Appendices

8.1 Recommendations for further work (Phase 2)

8.1.1 Development and validation (in-house) of a method to quantify 3-APA in retail foodstuffs

Develop / extend and validate a method to quantify 3-APA in foods based on LC-MS/MS of the sulphonamide derivative formed from the reaction with dansyl chloride.

8.1.2 Survey of 3-APA in retail foods

Quantify the distribution of 3-APA in a range of foodstuffs. Selection of foods for investigation might also include retained samples from the 2014 acrylamide-furan survey for which AA data is available. The latter might assist with subsequent interpretation of results for 3-APA and implications for the generation of AA.

Table 7. Suggested foods for analysis of 3-APA (and AA)

Foodstuff	Risk factors / Comments
Bread	No data.
Biscuits	No data. Selection of sweet and savoury samples
Breakfast cereals	Survey samples e.g. puffed wheat.
Chocolate	Relatively high amounts of 3-APA reported.
Cocoa	Relatively high amounts of 3-APA reported. Include samples of cocoa powders, drinking chocolate.
Coffee	Chlorogenic acid, HMF, thermal processing.
Crisps, including seasonal samples	Relatively high amounts of 3-APA reported after potato storage.
French fries from fast food outlets	Relatively high amounts of 3-APA reported after potato storage.
French fries for home cooking, fresh and frozen	Relatively high amounts of 3-APA reported after potato storage.
Olives	Chlorogenic acids, processing. No data.
Popcorn, sweet and savoury	Relatively high amounts of 3-APA reported
Popped potato snacks	“Pop chips”
Prunes	Chlorogenic acids, processing. No data.
Potatoes, raw	Retail samples taken in March and November to assess seasonal / storage effects

8.1.3 Effects of thermal processing on selected foods

Since thermal processes may have a significant impact on acrylamide, a limited investigation may be undertaken to consider the effects of secondary / thermal processing on the generation of 3-APA / AA. Suitable foodstuffs / processes for investigation might include: toasting of bread; frying / baking / reheating of potato products; grilling / microwaving of fresh cheese.

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