Status Report on Acrylamide in Potato Products

Teagasc, The National Food Centre

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1. Summary

Acrylamide is a toxin that can potentially occur in high concentrations in heated starchy foods especially potato products such as crisps and french fries. In model systems isotopic substitution studies have demonstrated that acrylamide is formed via the Maillard type reaction between the amino acid aspargine and a carbonyl source such as the reducing sugars glucose and fructose. Levels of acrylamide in cooked potato products are primarily influenced by the levels of reducing sugars in the product and this in turn is influenced by storage time, temperature and variety of potato used. During cooking acrylamide formation begins to occur at temperatures above 100℃ and increases up to temperatures of 220℃ but decreases thereafter due to thermal degradation of the compound. Risk assessment studies on acrylamide intakes have been conducted in a number of countries and mg/kg body weight daily intakes have been estimated to be between 0.2-0.8. Adequate analytical techniques exist for quantification of acrylamide in potato and are mainly based around liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) and gas chromatography mass spectrometry (GC-MS) techniques

2. Introduction

Since the discovery in 2000 by a group of Swedish researchers (Rosen and Hellas, 2002; Tareke et al., 2002) that acrylamide (Figure 1), a substance classified as a potential carcinogen (IARC, 1994), occurred in heated starchy foods at concentrations many times in excess of levels permitted in drinking water (0.5μg/L, WHO (2004)) there has been explosion of interest in this area. As a result, a number of excellent and extensive reviews covering issues such as acrylamide toxicity (Dearfield et al., 1988, 1995; LoPachin, 2004; Rudēn, 2004), mechanism of formation (Lingert, 2002),

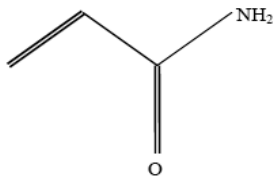

Figure 1. Structure of acrylamide

analysis of acrylamide (Wenzl et al., 2003) and general acrylamide reviews (Freidman, 2003; Tayemans, 2004;

The purpose of the present document is to provide a brief overview of the current thinking in relation to acrylamide in potato products. Most of the work published in the aftermath of the Swedish discovery falls broadly in to a small number of categories (although some studies span a number of the categories) these are:

- **Mechanistic and modelling studies**: The exact mechanism of acrylamide formation was unknown in the immediate aftermath of the discovery. However, major strides have been made in this area recently.
- **Monitoring of potential acrylamide precursors in foodstuffs**: Progress in mechanistic and modelling studies has lead to research into the levels of potential acrylamide precursors in uncooked foods.
- **Effect of processing on acrylamide formation:** The focus of these studies has been the development of processing protocols which limit acrylamide formation while maintaining product quality.
- **Population intake, risk assessment and toxicity studies**:

While there was some data available on acrylamide toxicity arising from the use of acrylamide in industrial applications, recent work has focussed on the potential toxicity of acrylamide from foodstuffs.

- **Methods for the analysis of acrylamide:** In order to determine acrylamide levels in the large variety of foodstuffs where it may potentially occur, reliable and sensitive analytical techniques for it's determination are essential.

FLAIR-FLOW debate: This was held at the NFC in December 2003.

Current study: Involves monitoring levels of acrylamide precursors in potato varieties commonly used for ware and processing and relating these levels to amounts of acrylamide formed in the cooked products prepared using a variety of cooking protocols (frying, roasting).

3. Mechanistic and modelling studies

In the early stages of investigations into the mechanism of acrylamide formation in heated foodstuffs, two routes to the formation of acrylamide were thought possible. Since acrylamide levels were high in fatty foods such as potato crisps and french fries the fatty acid oxidation product acrolein (CH_2=CH-CHO) was noted as a possible precursor and forming acrylamide through direct reaction with ammonia followed by oxidation to acrylamide (Gertz and Klostermann, 2002). Another possible route is via the reaction between reducing sugars and amino acids in the Maillard reaction. A number of recent mechanistic studies have shown that

the latter route is the most likely vehicle for acrylamide formation. Mottram *et al*. (2002) illustrated that significant quantities of acrylamide were formed when the amino acid asparagine and the reducing sugar glucose were reacted at 185°C in phosphate buffer. Asparagine is the most likely amino acid precursor as it possess an amide group attached to a chain of two carbon atoms and also occurs in significant quantities in potatoes and cereals (Brierley *et al*., 1992, 1996, 1997). Similarly, Stadler *et al*. (2002, 2004) reported that significant quantities of acrylamide were formed when equimolar amounts of glucose and asparagine were pyrolysed at 180°C. Biedermann *et al*. (2002a, 2003) also concluded that acrylamide formation resulted from the degradation of asparagine by reaction with a carbonyl source most likely from glucose and fructose. Beclaski *et al*. (2003) showed that ^{15}N-labelled glucose and asparagine in ratios similar to those found in potatoes produced ^{15}N-labelled acrylamide. Both Stadler *et al.* (2002) and Mottram *et al*. (2002) also postulated reaction pathways to acrylamide with the sugar asparagine adduct N-glycosylasparagine being suggested as a possible direct precursor of acrylamide under pyrolytic conditions. More recently this has been confirmed using pyrolysis gas chromatography/mass spectrometry (Py-GC/MS) and Fourier Transform Infra-Red (FTIR) spectroscopy (Yaylayan *et al*., 2003) and model studies (Stadler *et al*., 2004). Zyzak *et al*. (2003) using isotope substitution studies have elucidated the mechanism of acrylamide formation by confirming the presence of key intermediates such as a decarboxylated schiff base and 3-aminopropionamide. The acrolein route to acrylamide formation has been virtually

discounted as recent studies have confirmed that the addition of antioxidants did not affect acrylamide formation (Vattem and Shetty, 2003). In addition, real time monitoring of reducing sugars, asparagine and water contents in heated potato, wheat and rye systems have shown that losses are accompanied by increases in acrylamide formation and that this maximises near the end of the heating cycle (Elmore *et al*., In press). At the present time most available data points to the formation of acrylamide in foods by the route shown in Figure 2 (overleaf).

4. Monitoring of potential acrylamide precursors in foodstuffs.

The pioneering research of Mottram *et al*. (2002) and Stadler *et al*. (2002) strongly suggests that acrylamide formation in heated potato products results from the reaction of amino acids such as aspargine (and to some extent glutamine) and reducing sugars (glucose and fructose). This led to studies aimed at examining factors which affect the levels of these substances in raw potatoes. Noti *et al*. (2003) examined the effect of storage temperature and reconditioning at ambient temperature on sugar levels and the potential for acrylamide formation in potatoes. They recommended that storage below 8°C be avoided in order to avoid extensive acrylamide formation in the fried or roasted product. Similarly Biedermann et al. (2003) showed that in order to avoid excessive

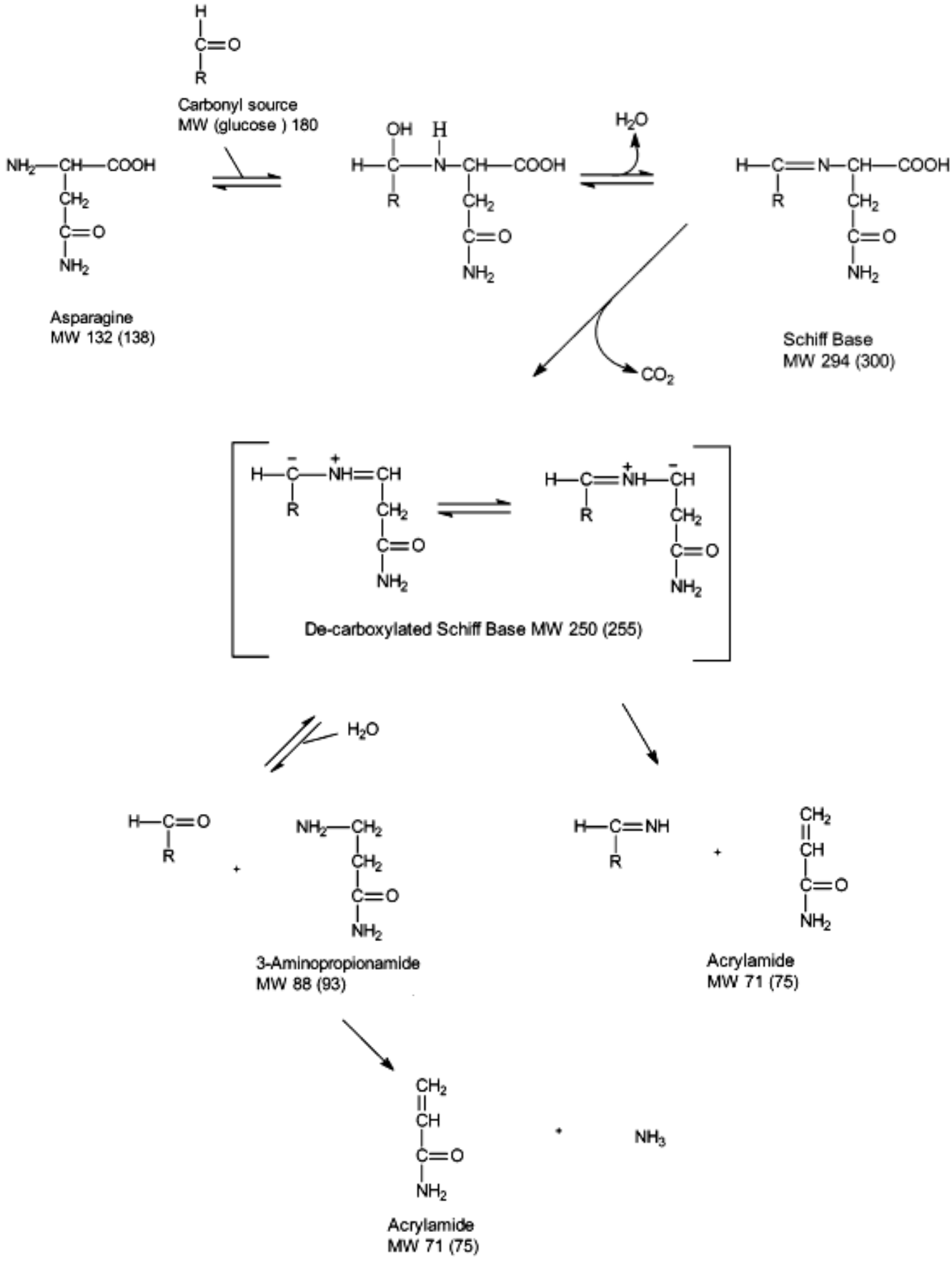

Figure 2. Probable route to the formation of acrylamide in starchy foods (Zyzak et al., 2003).

acrylamide formation while retaining adequate browning and flavour, potatoes with glucose and fructose contents in the range 0.2-1.0 g/kg fresh weight should be used for roasting and frying. Chuda *et al.* (2003) found that the acrylamide level of potatoes stored for 2 weeks post-harvest at 2°C was ten times higher than those held at 10°C and was highly correlated with both glucose and fructose levels in the tubers. Amrien *et al*. (2003) examined the effect of variety and farming systems on glucose, fructose and asparagine content and on subsequent acrylamide levels after frying. Variety had a significant effect on acrylamide formation which was primarily related to the reducing sugar content. In a separate study the Amrien *et al*. (2004b) found that the reducing sugar content was strongly correlated with acrylamide levels in the cooked product, whereas no correlation was found between acrylamide levels and free asparagine, or the pool of free amino acids. On a molar basis the mean content of aspargine was up to 5.6 times higher than that of glucose or fructose. Olsson *et al*. (2004) examined the effect of storage temperature, storage time and variety on the levels of aspargine, glutamine and reducing sugars in eight potato clones. They concluded that variety had a marked effect on all the components examined. In addition, storage at a low temperature (3°C) for nine months resulted in an increase in glucose and fructose levels. However, amino acid levels were not significantly affected by storage time. In a study on the effect of free amino acid and sugar levels on acrylamide formation in french fries, Becalski *et al*. (2004) found a significant correlation between acrylamide content and reducing sugar level. They also found that the presence of aspargine had a major effect on acrylamide levels although this was less significant than the

effect of reducing sugars. In conclusion, the levels of acrylamide in potato products are primarily influenced by the levels of reducing sugars in raw potatoes, and this in turn is influenced by storage time, temperature and variety of potato used.

5. Effect of processing on acrylamide formation in potato products

Potato products undergo a variety of processing steps prior to ingestion. The challenge for food processors and advisory/regulatory agencies is to establish processing protocols that limit the formation of acrylamide while maintaining finished product quality. A number of studies have shown that both the temperature and duration of heating have a significant influence on acrylamide levels. Rydberg *et al*. (2003) found that acrylamide levels increased in french fries as the oven temperature increased from 100-220°C, reaching a maximum level of 5000μg/kg. However, with prolonged heating at the maximum temperature acrylamide concentrations decreased presumably due to thermal degradation. Taubert *et al*. (2004) also demonstrated that heating potato slices with high to intermediate surface to volume ratios to temperatures above 180°C resulted in a rapid decrease in acrylamide levels. In contrast, Pedreschi *et al*. (2004) and Matthaus *et al*. (2003a,b) showed that increasing the frying temperature from 150-190°C resulted in a marked increase in acrylamide levels. No decrease in acrylamide formation was observed presumably because temperatures above 190°C and prolonged heating times (max .10 min) were not employed. Granda

et al. (2004) and Willams *et al*. (2005) also showed that both frying time and temperature increased acrylamide levels in potato chips. The former authors also showed that the use of low-temperature vacuum frying reduced acrylamide levels. It is worth noting that a number of authors have reported an inverse relationship between acrylamide contents and the moisture content of a foodstuff (Leung *et al*., 2003: Amrein *et al*., 2004). With particular reference to potato products Elmore *et al*. (In Press) noted that at moisture contents of 0.1-0.5% a linear inverse relationship existed between acrylamide levels in potato cakes and their moisture contents.

Techniques implemented prior to thermal processing also reduce acrylamide formation. Soaking or blanching of the raw product can reduce acrylamide content in the cooked product (Haase *et al*. 2003b; Grob *et al*., 2003; Pedreschi *et al*., 2004, 2005). However, Williams *et al*. (2005) found that inclusion of a water soak prior to frying had no effect on acrylamide formation. A further reduction in acrylamide levels can be achieved by immersion of the raw product in an acidic solution prior to cooking (Jung *et al*., 2003; Kita *et al*., 2004; Pedreschi *et al*., 2004). This method is particularly effective as it enhances the extraction of reducing sugars and amino acids from the product. Other pre-treatments shown to reduce acrylamide formation include addition of a flavanoid spice mix (Fernandez *et al*., 2003), use of asparginase to breakdown asparagine in the raw product (Zyzak *et al*., 2004) and the use of genetically modified potatoes having a reduced content of soluble sugars (Soyka *et al*., 2004).

6. Population intake, risk assessment and toxicity studies.

The toxicity of acrylamide was well known prior to the Swedish discovery and a number of excellent reviews are available regarding acrylamide toxicity (Dearfield *et al*., 1988, 1995; Freidman, 2003; Tayemans, 2004; LoPachin, 2004; Rudēn, 2004). Since the purpose the current document is to provide an overview of the information since the first identification of high acrylamide levels in foods, a detailed discussion of acrylamide toxicity is not provided. To date, risk assessment studies on potential acrylamide intake from foods have been published on populations in Belgium (Matthys *et al*., 2005), Sweden (Svensson *et al*., 2003, Mucci *et al*., 2003), Holland (Konings *et al*., 2003), Germany (Hilbig *et al*., 2004, Schettgen, 2002), Slovakia (Ciesarova *et al*., 2004), Japan (Maitani, 2004), Norway (Norwegian Food Control Authority, 2002), United Kingdom (FSA, 2005ab), Australia (Croft *et al*., 2004) and the USA (Javier, 2002, Petersen, 2002, DiNovi, 2004). Most of these studies have concentrated on assessing acrylamide intake in food products containing low to high levels of acrylamide and do not represent a complete dietary intake for the substance. Cooked potato products represents up to 35% of total daily intake of acrylamide (Norwegian Food Control Authority, 2002b). Appendix I lists a selection of population intakes for acrylamide based on published peer reviewed studies. Dietary intakes of acrylamide for the general population were estimated by FAO/WHO to be in the range of 0.3 to 0.8 μg /kg bw/day (Petersen, 2002). However, these dietary exposures are not directly comparable because of the different methods used for assessment i.e. different age groups, whole populations/ consumers of particular products using limited food groups rather than the whole diet. It is important to stress that it is still not clear whether or not acrylamide from food

represents a risk to public health and a recent population-based study in Sweden failed to find a link between dietary intake of acrylamide and cancer of the bowel, kidney and bladder (Mucci *et al.*, 2003). However, it is clear that the high profile nature of acrylamide in foodstuffs has raised public awareness to a level were further investigation is warranted (Gormley and Mee, 2003).

7. Analytical methodologies for determining acrylamide in potato products

Despite acrylamide being a relatively 'new' contaminant for food analysts, intensive method development and refinement have been carried out. An extensive review of analytical methods used for the determination of acrylamide has been published (Wenzl *et al*., 2003) followed by a substantial number of more recent papers. Appendix II lists methods that have been used to determine acrylamide in potatoes up to the present day and it is evident that GC-MS and LC-MS/MS are the most widely used methods. This was borne out in a recent proficiency study by the German Federal Institute on methods used for acrylamide determination where GC-MS and LC-MS/MS represented up to 94% of the methods (Clarke *et al*., 2003; Wenzl *et al*., 2004). GC-MS based methods fall into two categories those which include a derivitisation step which serves to increase selectivity and improve the volatilty of the compound and those without a derivitisation step. Bromination is the usual route to derivitisation for GC-MS with a variety of agents being used. GC-MS methods for the determination of acrylamide

without derivitisation require exhaustive extraction of the compound from the food matrix with extraction times extending to ten days in some cases (Pedersen and Olsson, 2003). LC-MS/MS methods generally do not require derivitisation. Quantification for both LC-MS/MS and GC-MS methods is generally achieved by the inclusion of either a [$^{13}C_3$]-acrylamide or [D_3]-acrylamide labelled internal standard during homogenisation of the sample. The use of MS detection as a method of quantification is probably related to the regulatory nature of the acrylamide problem. A method based on liquid chromatography-diode array detection (LC-DAD) and HPLC separation is available. However it may be suitable for more routine type analyses (Gökmen *et al*., 2004).

8. Acrylamide networks.

Owing to the high profile nature of the acrylamide debate a number of research networks have been established which provide valuable information on all aspects of the issue. Listed below are links to websites for some of the more extensive networks, however, a more comprehensive list is available from the Acrylamide Infonet Website

8.1 Acrylamide Infonet

http://www.acrylamide-food.org/

8.2 Food standards Agency (UK)

http://www.foodstandards.gov.uk/multimedia/pdfs/acrylamide_11Apr16May.pdf

8.3 The Heatox Project

http://www.slv.se/templatesHeatox/Heatox_default____8425.aspx

8.4 National Food and Administration (Sweden)

http://www.slv.se/templates/SLV_DocumentList____4089.aspx

8.5 Food Safety Risk Analysis Clearing house

http://www.foodriskclearinghouse.umd.edu/acrylamide.cfm

9. FLAIR-FLOW debate on on acrylamide in food December 2003.

A structured debate concerning acrylamide levels in food was held in December of 2003 in Dublin recently hosted by Dr. T.R. Gormley of The National Food Centre (Gormley and Mee, 2003) and was attended by 24 health professionals and three experts in the field. The areas of acrylamide formation, levels in different foods, safe levels of exposure, intakes by the population, methods of reducing the acrylamide content of foods during processing/cooking, the handling of the acrylamide issue in different countries and lastly advice to consumers was debated in depth. The debate was one of 72 debates being held on topical issues in 24 European countries in 2001-2003 as part of the EU-sponsored FLAIR-FLOW 4 dissemination project. The kernel of the debate programmes is that there are no lectures or presentations. Instead, the attendees are the proactive ones and cross examine the invited experts using a set of pre-arranged questions, i.e. a structured approach, over a two hour period. The consensus was that acrylamide in food is an emerging health concern based on current knowledge and that a careful watching brief should be maintained pending outcomes

from ongoing and future research. Below is list of the main outcomes of the debate:

- Extensive transnational epidemiological studies are required to clarify the risk to health posed by the ingestion of acrylamide from food, and to set a realistic maximum limit for acrylamide in food.
- In the interim, advice should be given to food processors, caterers and consumers on ways of reducing acrylamide levels in susceptible foods via preconditioning (e.g., blanching), reduced cooking temperatures and times, and through the use of emerging minimal processing techniques.

The importance of eating a balanced diet should continue to be advocated to the population as a whole, and to children and adolescents in particular, as should a reduction in the consumption of fried potato products counterbalanced by increased consumption of potatoes and potato products produced by boiling, steaming or microwaving.

10. FIRM sponsored project on acrylamide in potatoes and potato products

Beginning in January of last year a FIRM sponsored research project was established to investigate the factors influencing the formation of acrylamide in potatoes within an Irish context (RMIS No. 5265). The project has two main streams of investigation. The remit of the first stream is to investigate acrylamide formation in cooked potato/potato products. This part of the project is being conducted mainly in the National Food Centre involves monitoring

aspargine and reducing sugar contents in a range of potato varieties, stored under different conditions over eight months. In addition, tests for acrylamide precursors are being conducted on ware potato samples from supermarkets. Acrylamide levels will then be assessed using a LC-MS/MS method in a range of ware potatoes after frying. The effect of the cooking protocol i.e., frying time, blanching, microwave finishing on acrylamide levels will also be investigated. The other main thrust of the project involves conducting a risk assessment for acrylamide intake for various cohorts of the Irish population. This part of the project is being conducted in the Biosystems Engineering Department of University College Dublin. It will involve developing a quantitative risk assessment model for acrylamide intake from potato products and using information on Irish consumption of potato products by different cohorts (sex, age groupings) estimate annual individual intake of acrylamide for the various population subgroups. The project tasks are as follows:

- **Task 1**- To conduct a literature survey on acrylamide in cooked potatoes/potato products and in other relevant food products and on factors influencing it's formation (NFC).
- **Task 2**- To test the content of asparagine, reducing sugars and acrylamide in potatoes/cooked potato products (NFC).
- **Task 3**- Risk assessment of acrylamide intake from Irish potato products (UCD).
- **Task 4-** Dissemination of results (NFC).

11. Conclusions

Significant progress on the occurrence and quantification of acrylamide in potato products has been made over the past 3 years, as reflected by the numerous publications and national and international workshops on the subject. This rapid pace of developments is mainly attributable to the co-ordinated and collaborative efforts of all of those concerned: the food industry, academia, private/enforcement laboratories, and national authorities. The majority of the information gaps identified since the findings in early 2002 on the occurrence of acrylamide in foods and exposure assessments have been addressed, and public databases have been established by several authorities. Today the performance of analytical methods for the determination of acrylamide in potato products and other more difficult matrices is adequate. Investigations into acrylamide precursors in potatoes and the effect of processing on acrylamide levels have provided better knowledge of the key parameters that influence acrylamide formation. However, despite these intensive efforts, only marginal reductions have been achieved by the food industry, and any further progress will entail long-term studies at the primary production level. Any measures devised to reduce exposure to acrylamide in commercial foods must be carefully assessed in terms of food safety and quality. To date a poorly addressed concern is the formation of acrylamide in foods prepared by consumers in the home, and more guidance on this by national authorities is warranted

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Appendix I. Esitmated daily dietary intakes of acrylamide.

Authors	Country	Intake (μg /kg bw/ day)
Svennson *et al*. 2003	Sweden	0.50
Matthys *et al*. 2005	Belgium	0.59[a]
Konings *et al*. 2003	Holland	0.48
Di Novi et al. 2004	USA	0.43
FSA, 2005	UK	0.54
Hilbig *et al*. 2004	Germany	0.21-0.43[b]
Norwegian Food Control Authority, 2002	Norway	0.33-0.36
Croft *et al*. 2004	Australia	0.40-0.50

[a] *Refers to Flemish adolescents only*

[b] *Refers to children from the ages of 1-18*

Appendix II. Analytical Methods for the quantification of acarylamide in cooked potato products

Authors	Extraction Protocol	Internal Standard	Derivitised	Method	Column Parameters	Detection Methods
Tareke *et al.* 2002	Homogenisation in H_2O with IS followed by centrifugation	$[^{13}C_3]$-acrylamide	No	LC-MS/MS	Column-Hybercarb (50x2.1mm, 5μm), Mobile Phase: H_2O, Flow Rate: 0.2 ml min^{-1}	MS-MS: *m/z* 72>54 (acrylamide), *m/z* 75-58 $[^{13}C_3]$-acrylamide
Becalski *et al*. 2003	Grinding with H_2O + CH_2Cl_2 and IS followed by centrifugation	$[^{13}C_3]$-acrylamide or $[D_3]$-acrylamide	No	LC-MS/MS	Column-Hybercarb (50x2.1mm, 5μm), Mobile Phase: 15% MeOH in aqeous ammonium formate (1m) , Flow Rate: 0.175 ml min^{-1}	MS-MS: *m/z* 72>54 (acrylamide), *m/z* 75-58 $[^{13}C_3]$-acrylamide
Ahn *et al*. (2002)	Extraction in hot H_2O for 2hr followed by vacuum filtration	Methylacrylamide, $[^{13}C_1]$-acrylamide or 2,2,3-$[D_3]$-acrylamide	No	LC-MS/MS	Column-Primishpere C18HC (250x3.2mm, 5μm), Mobile Phase: 1% CH_3COOH in H_2O, Flow Rate; 0.5 ml min^{-1}	MS-MS: *m/z* 72>54 (acrylamide), 2,2,3-$[D_3]$-acrylamide (not specified)

Rosen and Hellas, (2002)	Homogenisation in H_2O with IS followed by centrifugation	$[D_3]$-acrylamide	None	LC-MS/MS	Column-Hybercarb (50x2.1mm, 5μm), Mobile Phase: H_2O, Flow Rate: 0.4 ml min^{-1}	MS-MS: *m/z* 72>54 (acrylamide), *m/z* 75-58 $[^{13}C_3]$-acrylamide

Appendix II. Analytical Methods for the quantification of acarylamide in cooked potato products (contd)

Authors	Extraction Protocol	Internal Standard	Derivitised	Method	Column Parameters	Detection Methods
Hofler *et al*. (2002), Cavali et al. (2003)	Homogenisation with accelerated solvent extracted with H_2O or formic acid at 80°C	None	None	LC-MS/MS	Column-IonPac ICE-ASI (150x2.1mm, 3μm), Mobile Phase: 10% MeOH in H_2O, Flow Rate: 0.1 ml min^{-1}	MS-MS: *m/z* 72>54 (acrylamide), *m/z* 75-58 [[$^{13}C_3$]-acrylamide
Jezussek *et al*. 2003	Homogenisation in H_2O with IS followed by centrifugation and defatting with n-hexane	[$^{13}C_3$]-acrylamide	2-Mercapto benzoic acid	Stable Isotope LC-MS	Column-Luna Phernyl-Hexyl (250x4.6mm, 5μm), Mobile Phase: Acetonitrile:CH_3COOH, Flow Rate: 0.8 ml min^{-1}	MS: m/z 226 (acrylamide), *m/z* 229 [$^{13}C_3$]-acrylamide
Roach *et al*. 2003	Homogenisation in H_2O with IS followed by centrifugation	[$^{13}C_3$]-acrylamide	None	ESI-LC-MS/MS	Column-Synergi Hrdro-RP 80A (250x2.0mm, 4μm), Mobile Phase: Acetonitrile:0.5% MeOH/0.1%CH_3COOH in H_2O,	MS-MS: m/z 72>55 (acrylamide), *m/z* 75-58 [$^{13}C_3$]-acrylamide

Calbiani *et al*. 2004	Vortexing in 0.1% formic acid with IS followed by centrifugation	[$^{13}C_3$]-acrylamide	None	ESI-LC-MS/MS	Column-Luna C18(2) (250x2.1mm, 5μm), Mobile Phase: 0.1% formic acid:MeOH (99.5:0.5), Flow Rate: 0.2 ml min^{-1}	MS-MS: *m/z* 72>55 (acrylamide), *m/z* 75-58 [$^{13}C_3$]-acrylamide

Appendix II. Analytical Methods for the quantification of acarylamide in cooked potato products (contd)

Authors	Extraction Protocol	Internal Standard	Derivitised	Method	Column Parameters	Detection Methods
Riediker and Stadler, 2003	Homogenisation in H_2O with IS followed by extraction into acetonitrile and SPE clean-up	[$^{13}C_3$]-acrylamide	None	Stable Isotope LC-ESI-MS/MS	Column-Shoda RSPAK DE-613 polymthacrylate (150x6.0mm), Mobile Phase: 0.1% formic acid:MeOH (6:4), Flow Rate: 0.75 ml min^{-1}	MS-MS: *m/z* 72>55 (acrylamide), m/z 75-58 [[$^{13}C_3$]-acrylamide
Young *et al*. 2004	Crushing in NaCl (2M) followed by mixing centrifugation SPE clean-up	[D_3]-acrylamide	None	LC-MS	Column-Atlantis d C_{18} (150x2.1mm), Mobile Phase: 0.1% formic acid in water Flow Rate: 0.2 ml min^{-1}	MS: *m/z* 72 (acrylamide), *m/z* 75 [$^{13}D_3$]-acrylam

Ahn *et al*, 2002	After homgenisation extracted with hot H_2O (80°C) for 2 hr	methylacrylamide	bromination	GC-MS	Column-DB 17 (30mx0.25mm, 0.25μ i.d.), Heating programme: 85°C (1 min)→175°C @ 25°C min^{-1} →250 (5min) @ 40°C min^{-1}	MS- *m/z* = 106,108,150,152 (acrylamide), *m/z* = 120, 122 methylacrylamide
Biedermann *et al*. 2002	Homogenisation (with IS) in H_2O then extraction with 1-propanol and centrifugation	IS 1-(CH_3)acrylamide + [D_3]-acrylamide	None	GC-MS	Column- Carbowax 20M (10mx0.25mm (i.d), 0.4μm film thickness	MS- *m/z* 72 (acrylamide), *m/z* 75, 86 (IS 1) *m/z* = 88 (IS 2)

Appendix II. Analytical Methods for the quantification of acarylamide in cooked potato products (contd)

Authors	Extraction Protocol	Internal Standard	Derivitisation	Method	Column Parameters	Detection Methods
Swiss Federal Office of Public Health, (2002)	Homogenisation (with IS) in H_2O with SPE clean-up and evaporation	$[D_3]$-acrylamide	None	GC-MS	Column- DB-WaX (30mx0.25mm (i.d), 0.25μm film thickness, Heating programme: 50°C (3.0 min)→240°C @ 15°C min^{-1} (9 min)	MS- m/z 71 (acrylamide), m/z 74 $[D_3]$-acrylamide
Gertz and Klostermann, (2002)	Ezymic digestion with clara diastase (18 hr) followed by centrifugation and filtration	$[D_3]$-acrylamide	Potassium Bromide	GC-MS	Column- J & W (30mx0.25mm (i.d), 0.2μm film thickness, Heating programme: 50°C (30 sec) → 270°C @ 8°C min^{-1} (10 min)	MS- m/z 106 (acrylamide), m/z 109 ($[D_3]$-acrylamide)
Hoenicke *et al*. 2004	Deffatting, addition of H_2O then extraction into hexane and sonication	$[D_3]$-acrylamide	None	LC-MS	Column: Merck LiChrospher 100 CN column (250mm × 4mm I.D., μm) mobile phase, 50% acetonitrile in 1% acetic acid	*MS: m/z* 72 > 72, 72 > 55, (acrylamide) *m/z* 75 > 75, 75 > 58, and 75 > 44 $[D_3]$-acrylamide

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