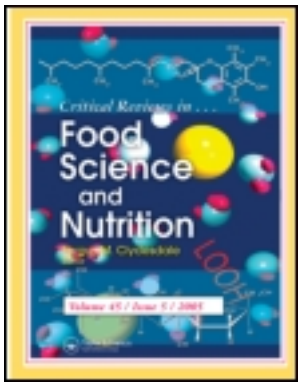

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A Review of Acrylamide: An Industry Perspective on Research, Analysis, Formation, and Control

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Acrylamide is a synthetic monomer with a wide scope of industrial applications, mainly as a precursor in the production of several polymers, such as polyacrylamide. The main uses of polyacrylamides are in water and wastewater treatment processes, pulp and paper processing, and mining and mineral processing. The announcement by the Swedish National Food Administration in April 2002 of the presence of acrylamide predominantly in heat-treated carbohydrate-rich foods sparked intensive investigations into acrylamide, encompassing the occurrence, chemistry, agricultural practices, and toxicology, in order to establish if there is a potential risk to human health from the presence of this contaminant in the human diet. The link of acrylamide in foods to the Maillard reaction and, in particular, to the amino acid asparagine has been a major

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step forward in elucidating the first feasible chemical route of formation during the preparation and processing of food. Other probably minor pathways have also been proposed, including acrolein and acrylic acid. This review addresses the analytical and mechanistic aspects of the acrylamide issue and summarizes the progress made to date by the European food industries in these key areas. Essentially, it presents experimental results generated under laboratory model conditions, as well as under actual food processing conditions covering different food categories, such as potatoes, biscuits, cereals, and coffee. Since acrylamide formation is closely linked to food composition, factors such as the presence of sugars and availability of free amino acids are also considered. Many new findings that contribute towards a better understanding of the formation and presence of acrylamide in foods are presented. Many national authorities across the world are assessing the dietary exposure of consumers to acrylamide, and scientific projects have commenced to gather new information about the toxicology of acrylamide. These are expected to provide new scientific knowledge that will help to clarify whether or not there is a risk to human health from the consumption of foods containing low amounts of acrylamide.

Keywords acrylamide, analysis, foods, formation, industry, Maillard reaction, processing

INTRODUCTION

In April 2002, the Swedish National Food Administration and the University of Stockholm jointly announced that certain foods that are processed/cooked at high temperatures contain relatively high amounts of acrylamide (Swedish National Food Administration, 2002), following on an earlier rat feeding study that demonstrated a link of acrylamide in fried animal food to specific hemoglobin adducts (Tareke et al., 2000). Exposure to acrylamide causes damage to the nervous system in humans and animals (Lopachin and Lehning, 1994; Tilson, 1981), and acrylamide is also considered a reproductive toxin (Costa et al., 1992; Dearfield et al., 1988) with mutagenic and carcinogenic properties in experimental mammalian *in vitro* and *in vivo* systems (Dearfield et al., 1995).

These findings have attracted considerable interest worldwide, because acrylamide has been classified as "probably carcinogenic to humans" by the International Agency for Research on Cancer (IARC, 1994). The Swedish findings were quickly confirmed by a number of government agencies through website notifications (Food Standards Agency, UK, 2002), and the potential health risk of acrylamide in food was consequently considered (Scientific Committee on Food (SCF) opinion, 2002). Following these deliberations, all available data on acrylamide have been reviewed at an international level (e.g., by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations (UN) (FAO/WHO Consultation, 2002), Joint Institute for Food Safety and Applied Nutrition/National Center for Food Safety and Technology (JIFSAN/NCFST) Workshop (JIFSAN/NCFST Workshop, 2002)) by expert Working Groups, identifying and listing a number of research gaps and priorities. Once addressed, these will allow a better assessment of the potential health risks associated with this unexpected finding.

Thermal processes in the production of foodstuffs are generally complex, and the initial results on acrylamide content did not seem to indicate a common pattern, except that carbohydrate-rich foods seemed to generate relatively more acrylamide. Another important aspect is that low water content seems important for the reaction(s), and acrylamide was not detected in boiled foods containing starch (e.g., potatoes). Several months after the Swedish announcement, a number of research groups simultaneously discovered that acrylamide is formed during the Maillard reaction, and the major reactants leading to the formation of acrylamide are sugars and the amino acid asparagine (Becalski et al., 2002; Mottram et al., 2002; Sanders et al., 2002; Stadler et al., 2002; Weisshaar and Gutsche, 2002). From the outset, the food industry as a major stakeholder has taken a responsible, collaborative, and transparent position on this issue. The European food industry, represented by the CIAA (Confederation of the food and drink industries of the EU), has been working with the European Commission and Member States. This includes (a) the co-ordination of research; (b) procuring and making available analytical data via the CIAA database (now handed over to the European Commission's Directorates General Joint Research Center (JRC) and combined with the JRC's Institute for Reference Materials and Measurements (IRMM) database); (c) leading the research in the development of analytical methods; (d) contributing significantly in understanding how acrylamide is formed in foodstuffs (Sanders et al., 2002; Stadler et al., 2002). Intensive research in these key areas is ongoing: although significant progress has been made already, many gaps in the science are evident and must be addressed. For example, in the analytical field the performance of current methods used for the determination of acrylamide is not adequate for the more "difficult" food matrices, such as cocoa, coffee, and high salt flavorings. Having sensitive and robust methods that provide reliable data in the different food categories is of crucial importance for intake assessments. Staple foods, such as bread that contains acrylamide only at trace amounts (in the low part-per-billion range), may, nevertheless, contribute significantly to the overall dietary intake.

Although a favored mechanism for the formation of acrylamide is the Maillard reaction, involving the condensation of the amino acid asparagine and a carbonyl source (e.g., sugars), fundamental studies have very recently revealed new avenues, identifying acrylic acid as a potential precursor of acrylamide in model systems (CIAA/UK FSA/Dutch VWA Workshop, 2003). Once this fundamental knowledge has been acquired, concrete studies can be devised that include kinetic modelling (formation

over temperature/time, competitive reaction kinetics with amino acids and sugars) and identifying the rate limiting steps under actual food processing conditions. Measures can then be devised to attempt to reduce acrylamide in food products. This will, however, necessitate extensive individual trials for each food category.

This report summarizes the current state of knowledge and the collective efforts being made by the European Food Industry in acrylamide research, focusing on the analytical, mechanistic, and mitigation aspects. Furthermore, the progress and complexity of the research is highlighted in a number of different products, indicating the challenges and constraints faced by the industry in finding appropriate and practical solutions to this concern.

ANALYSIS OF ACRYLAMIDE IN FOOD

This section provides an overview of the major analytical approaches taken by laboratories to determine acrylamide in cooked foods, highlighting what has been achieved so far, the challenges remaining, and how these should be prioritized with the available resources.

Numerous methods have been developed in the past years to determine the acrylamide monomer, especially in water, biological fluids, and non-cooked foods (sugar, field crops, mushrooms). The majority are classical methods based on high performance liquid chromatography (HPLC) or gas chromatographic (GC) techniques (Bologna et al., 1999; Castle, 1993; EPA, 1996; Tekel et al., 1989). However, because of the complexity of food matrices, these methods as such do not suffice for the analysis of acrylamide in processed or cooked foods at low levels. Particularly, they lack selectivity and the additional degree of analyte certainty required to confirm the presence of a small molecule, such as acrylamide, in a complex food matrix.

The first method pertaining to the analysis of acrylamide in different cooked and processed foods was reported in May 2002 and is based on the use of isotope dilution liquid chromatography-mass spectrometry (LC-MS) (Rosén and Hellenäs, 2002). Since then, several analytical methods dealing with the analysis of acrylamide in cooked foods have been published in peer reviewed journals or presented at international scientific meetings (for a review, see Wenzl et al., 2003). These methods are based mainly on MS as the determinative technique, coupled with a chromatographic step either by LC (Ahn et al., 2002; Becalski et al., 2003; Hartig et al., 2002; Höfler et al., 2002; Gutsche et al., 2002; Nemoto et al., 2002; Tareke et al., 2002), or GC, the latter either after derivatization of the analyte (Gertz and Klostermann, 2002; Höfler et al., 2002; Ono et al., 2003; Tareke et al., 2002), or, in a few cases, analysis of the compound directly (Biedermann et al., 2002a; Tateo and Bononi, 2003; Swiss Federal Office of Public Health, 8.10.2002). The Working Group on Analytical Methods that convened during the recent meeting on acrylamide (JIFSAN/NCFST, 2002), the European Workshop on analytical methods for the determination of acrylamide in food products (Joint European Commission Workshop, 2003), and Clarke et al. (2002) concluded that the majority of laboratories involved in acrylamide analysis use either GC-MS or LC-MS methods. The advantage of the LC-MS-based methods is that acrylamide can be analyzed without prior derivatization (e.g., bromination), which considerably simplifies and expedites the analysis.

Methods based on Gas Chromatography-Mass Spectrometry

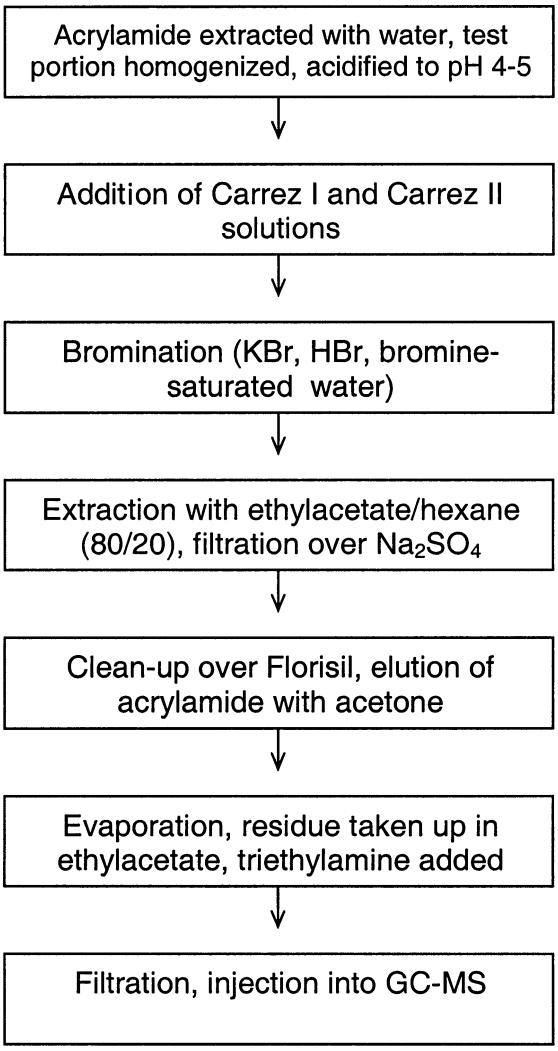
Assays employing GC-MS techniques are either based on bromination of the analyte or direct analysis without derivatization. The latter approach is less laborious and in both reported cases, employs liquid–liquid extraction of the analyte. In the method reported by Biedermann et al. (2002a), the determinative step is either positive ion chemical ionization in the selected ion monitoring (SIM) mode or electron impact ionization, achieving a level of detection (LoD) of around 50 and <10 μg/kg, respectively, for potato products. Better sensitivity (level of quantitation, LoQ $= 5$ μg/kg) can be achieved in the tandem (MS/MS) mode using a high resolution mass spectrometer.

Although more tedious, the bromination of acrylamide to 2,3-dibromopropionamide has multiple advantages, which include (a) improved selectivity, (b) increased volatility, (c) removal of potentially interfering co-extractives, and (d) better sensitivity. Usually the ions m/z 150/152 $[CH_2{-}CHBr{-}CONH_2]^+$ and m/z 106/108 $[CH_2{=}CHBr]^+$ are monitored in the selected ion monitoring (SIM) mode. Some analysts, however, choose to convert the rather labile di-bromo derivative to 2-bromopropenamide by treatment with triethylamine. This additional step avoids the risk of dehydrobromination in the injector or the ion source of the MS and has no impact on the selectivity or sensitivity of the method. In this case, the ions m/z 149/151 $[CH_2{=}CBr{-}CONH_2]^+$ and m/z 106/108 are chosen in the SIM mode. Therefore, GC-MS after bromination is probably the best choice for the analysis of acrylamide in foods necessitating a detection level at or <10 μg/kg. A typical flow chart illustrating the individual steps in sample preparation and extraction for GC-MS analysis is shown below (Pittet et al., 2004) (Scheme 1).

A further advantage of this technique is that a relatively simple benchtop, GC-MS, can be employed for acrylamide analysis. Application of GC-MS/MS or coupling to a high resolution MS would even further lower the detection limit for certain foods, approaching the range of 1–2 μg/kg.

Methods based on Liquid Chromatography-Mass Spectrometry

The first LC-MS method for acrylamide in cooked foods was developed in early 2002 by Rosén and Hellenäs to verify the initial results procured in Sweden by GC-MS. The method essentially entailed extraction of the analyte with water, centrifugation, solid phase extraction over a Multimode (Isolute®) cartridge, filtering over a 0.22 μm syringe filter, and



Scheme 1 Sequence of steps for the analysis of acrylamide in foods by GC-MS.

subsequently, over a centrifuge spin filter (cut-off 3kDa). Due to the low molecular weight of acrylamide (71 g/mol) and also its low mass fragment ions, confirmation of the analyte can be achieved with a three stage mass spectrometer (monitoring of more than one characteristic mass transition). However, acrylamide is a very polar molecule with poor retention on conventional LC reversed-phase sorbents (Ahn et al., 2002), and despite the use of a tandem mass spectrometer, more effort must, in most cases, be placed on the clean-up steps to avoid interference from co-extractives. Of the LC-MS methods communicated at different expert meetings, workshops, or published in the scientific literature, most are making use of solid-phase extraction (SPE) during the clean-up step. Acrylamide is difficult to bind actively to any of the conventional sorbents, but the major advantage of SPE is the retention of interfering matrix constituents. Therefore, enrichment or concentration of the analyte remains a challenge, and relatively low absolute recoveries have been recorded in the range of 62–74% in breakfast cereals and crackers, respectively (Riediker and Stadler, 2003).

Since the initial Swedish announcement, food industry laboratories have worked intensively on the development of LC-MS based methods to determine acrylamide in processed and cooked foods (Riediker and Stadler, 2003; Roach et al., 2003; Zyzak et al., 2003). Similar to the experiences of private and official food control laboratories, problems have been encountered in the analysis of difficult matrices due to interfering compounds in the characteristic acrylamide transitions (either for the internal standard or the analyte). A promising approach is to extract the analyte into a polar organic solvent, such as ethyl acetate. Sanders et al. (2002) have employed ethyl acetate to extract acrylamide from the aqueous phase (removing interfering constituents such as salt, sugars, starches, amino acids, etc.). The ethyl acetate extract can then be concentrated and analyzed by either LC-MS or GC-MS. In most cases, the LoD is significantly lowered, even approaching 10 μg/kg (Zyzak et al., 2003). Similarly, an ethyl acetate extraction step can also be included after the SPE clean-up, providing a significant improvement in sensitivity, especially for more difficult matrices, such as cocoa powder and coffee (Figure 1).

Continuous progress is being made in optimizing LC-MS methods and reducing the quantification limits. Recently, Jezussek and Schieberle (2003a and 2003b) have reported a promising method by derivatizing acrylamide with 2-mercaptobenzoic acid to the thioether and measuring the resulting adduct with a single-stage mass spectrometer. This method achieves an improvement in sensitivity of approximately 100-fold versus the non-derivatized analyte. Such approaches may potentially form the basis for the development of even more simple LC-UV methods for the determination of acrylamide in foods.

On-Line Methods—Proton Transfer Reaction Mass Spectrometry

Proton transfer reaction mass spectrometry (PTR-MS) has been shown to be a suitable method for rapid and on-line measurements of volatile compounds of headspace samples (Lindinger et al., 1993, 1998; Yeretzian et al., 2002). It combines a soft, sensitive, and efficient mode of chemical ionization, with a quadrupole mass filter. The headspace gas is continuously introduced into the drift tube, which contains a buffer gas and a controlled ion density of H_3O^+. Volatile organic compounds (VOCs) that have proton affinities larger than water are ionized in the drift tube by proton transfer from H_3O^+, i.e., $VOC + H_3O^+ \rightarrow [VOC + H]^+ + H_2O$. The protonated VOCs are extracted from the drift tube by a small electric field and mass analyzed in the quadrupol mass spectrometer. The four key features of PTR-MS can be summarized as follows: (a) it is fast, and time dependent variations of headspace profiles can be monitored with a time resolution of about 0.1 s; (b) the volatiles are not subjected to work-up or thermal stress and little fragmentation is induced by the ionization step, hence, mass spectral profiles closely reflect genuine headspace

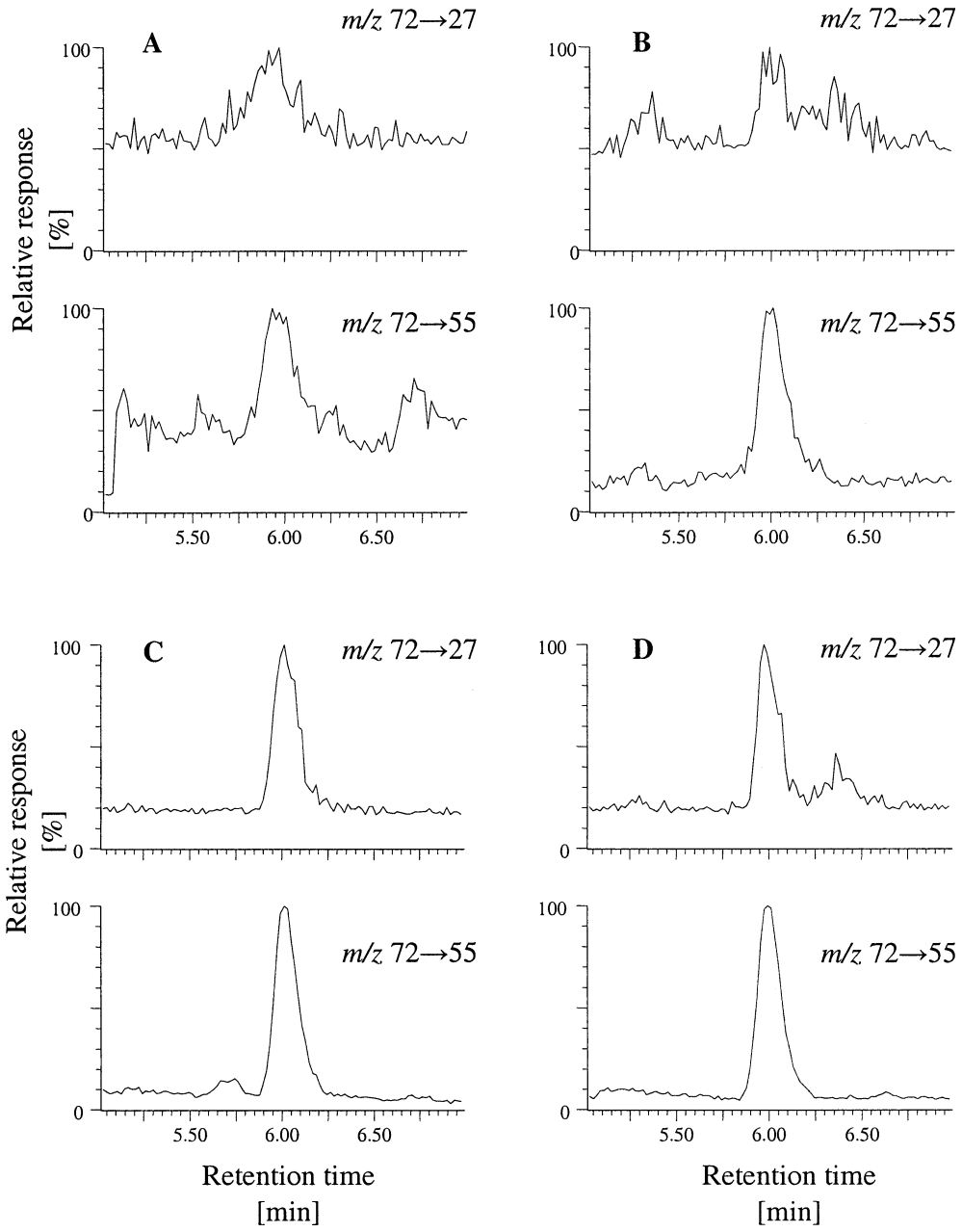


Figure 1 SRM traces (m/z 72 → 55, 72 → 27) obtained from a sample of cocoa powder (left panels), and roast and ground coffee (right panels). Sample preparation encompassed an SPE clean-up step (A, B) and, in addition, a liquid-liquid extraction using ethyl acetate (C, D). Retention time of acrylamide at ∼6 m.

distributions; (c) mass spectral intensities can be transformed into absolute headspace concentrations; (d) it is not invasive. All these features make PTR-MS particularly suited to investigate fast dynamic processes, such as formation of aroma and volatile contaminants in Maillard reactions.

The applicability of the PTR-MS approach for monitoring on-line the formation of acrylamide was evaluated in real food systems using thermally treated potatoes as an example (Pollien et al., 2003). The mass trace at m/z 72 indicated the presence of acrylamide in the headspace obtained by heating potato at 170°C. The mass at m/z 72 was found to be homogeneous, without interference with other volatile compounds, using an off-line coupling method (Figure 2). Retention index and EI mass spectrum were identical with those of the acrylamide reference compound, and only one peak with the mass at m/z 72 was detected by PTR-MS. The EI spectrum of the compound eluting 49.6 m was conclusively identified by the Wiley EI database as acrylamide.

The formation of acrylamide on heating dried potato slices at 170°C for 70 m showed a rapid initial increase, followed by a broad maximum after 6–10 m of reaction time, and subsequently, with a slow decline of the curve (Figure 3). However, the amounts of acrylamide in the headspace were very low compared to the Maillard model systems (data not shown) (Pollien

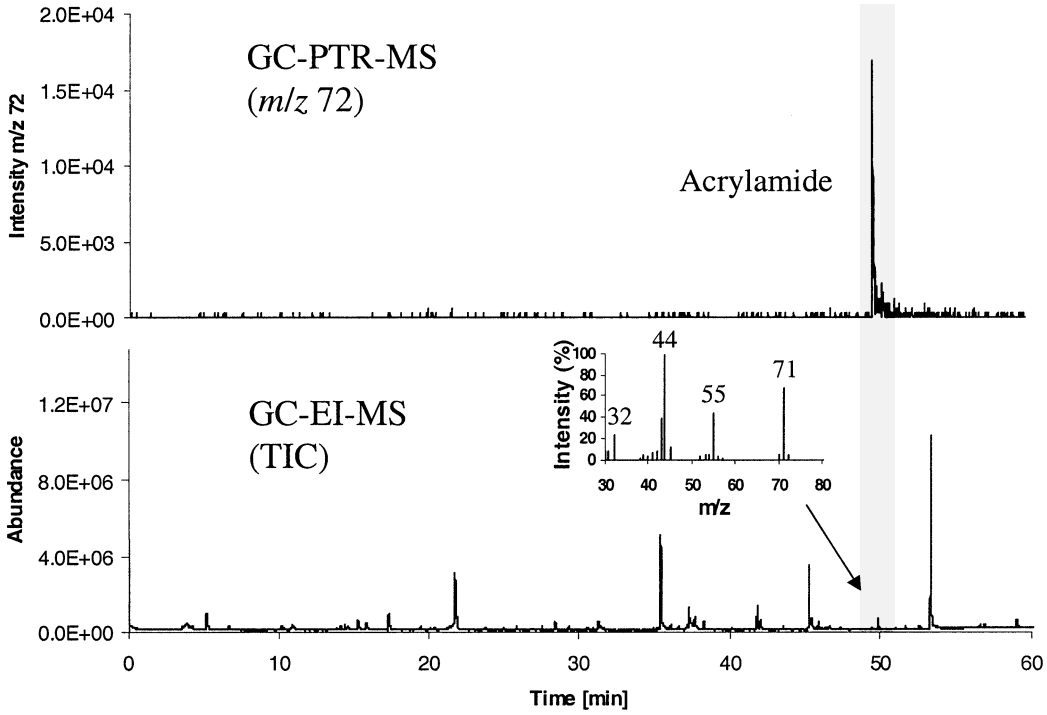


Figure 2 Identification of acrylamide by GC—EI-MS/PTR-MS, from dried potato at 170°C. The bottom trace shows the total ion current (TIC) in GC EI-MS. The snapshot shows the EI-MS of the compound eluting at 49.6 m. The top trace represents the corresponding GC PTR-MS trace at m/z 72, obtained simultaneously with the TIC. The PTR-MS ion signal at m/z 72 is exclusively related to the GC peak eluting at 50 m and corresponds to acrylamide.

et al., 2003), which is most likely due to the lower concentration of the precursors (reducing sugars and asparagine), but also to the high polarity and low volatility of acrylamide.

According to literature data (Martin and Ames, 2001), fresh potato contains about 1000 mg/kg of free asparagine. Taking into account the high water content of ca. 80% (Souci et al., 2000), an estimated 2.5 mg (19 μmol) of asparagine was available in 0.5 g dried potatoes for generating acrylamide. Despite the low precursor amounts in the experiment with potatoes, these data show that PTR-MS is sufficiently sensitive to monitor the formation of acrylamide under food processing conditions.

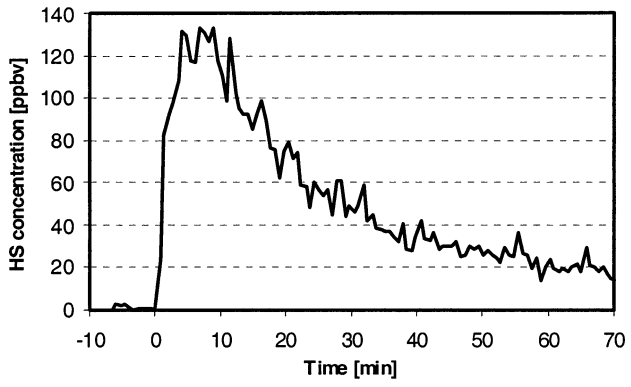


Figure 3 PTR-MS time-intensity profile for the formation of acrylamide from dried potato slices at 170°C.

Interlaboratory Proficiency Tests

Shortly after the Swedish announcement of the acrylamide issue, numerous small-scale interlaboratory tests were organized to assess the reproducibility of the applied methods, (e.g., US National Food Processors Association (NFPA) Swiss Federal Office of Public Health) and compare the data generated by different laboratories on more "simple" matrices. More extensive proficiency tests were administered by the UK's Central Science Laboratory (CSL) according to the Food Analysis Performance Assessment Scheme (FAPAS) in crisp bread (Clarke et al., 2002), revealing relatively large tolerances for this matrix, i.e., 836–1590 μg/kg (acceptable z-score = 2). The interlaboratory scheme organized recently by the German *Bundesinstitut für Risikoforschung* (BfR) showed similar tolerances, i.e., for crisp bread 81–286 μg/kg ($CV_R = 28\%$) and mashed potato (3.55 − 11 mg/kg, $CV_R = 25.6\%$) (Faul et al., 2002). Greater problems were encountered with cocoa powders, which did not provide acceptable performance, possibly attributable to the different extraction techniques used and the difficulty of many laboratories in reliably determining acrylamide in this complex matrix. Thus, the acceptable range is far too wide in the food products so far tested, even for relatively simple matrices. In this context, the use of more stringent z-scores to "filter" out the less proficient laboratories and thereby tighten the acceptable range in order to improve the quality of acrylamide measurements, especially in the food categories where the matrices are relatively simple, is warranted. This consideration should be taken seriously,

especially since reliable data describing acrylamide contents in foodstuffs will be needed for use in estimating exposure and risk assessment.

Constraints in the Current Methodologies

A challenge remaining today is the development of reliable and robust methods for difficult food matrices, such as cocoa powders, coffee, and high salt flavorings. An additional concern is the issue of sampling, which must be statistically representative for a product and, thus, demands adequate procedures to be put in place. Apparently, large variability of the acrylamide concentrations within a given food product brand, i.e., batch-to-batch, has been demonstrated (Table 1). A Recent study has attributed high variation within a sample to extraction problems of the current methods (Pedersen and Olsson, 2003). In this study, the use of a Soxhlet extraction technique (methanol) over 10 days considerably increased the amount of acrylamide extracted from French fries (14.5 mg/kg versus 2.28 mg/kg). However, the conditions of Soxhlet extraction of potato chips with methanol liberates sufficient quantities of free asparagine and sugars to form acrylamide when directly analyzed by GC-MS. Further, the conditions of extraction under low moisture may have contributed to the formation of the early precursor, i.e., Schiff base of available free asparagine and reducing sugar. The Schiff base is in equilibrium with the *N*-glycoside, known to be unstable, releasing acrylamide at relatively low temperatures. Thus, continuous extraction as described by Pedersen and Olsson may favor the Maillard reaction and potentially lead to *in situ* formation of acrylamide.

These difficulties and uncertainties need to be considered in the context of signal values and the dynamic minimization principle applied in Germany (Künast, 2002). Although this measure has no enforcement status, a certain flexibility in the range must be accepted, taking into account method reproducibility, errors due to sampling, and natural variation within a product brand.

In summary, mainly two methods of analysis (LC-MS or GC-MS) are used by laboratories world wide, and based on the early indications of proficiency tests, it is difficult to say that one is more reliable than the other. Limits of quantification range from 30–50 μg/kg for LC-MS down to 10–30 μg/kg for GC-MS.

Table 1 Within-product variation of acrylamide levels (Data for crisp bread and potato crisps are taken from Weisshaar and Gutsche, 2002)

Samples	Acrylamide (μg/kg)	Average	RSD (%)
Crisp Bread*	1460, 816, 1212, 1714, 1627	1366	27
Potato crisps**	796, 777, 1470, 1530	1143	36
Chocolate bar	86, 55, 146, 73, 116, 50	88	42
Cereal A***	205, 556, 401, 202, 267, 230, 146	287	50
Cereal B***	365, 398, 268, 499, 372, 180, 196, 164	305	40

Signal values *610 μg/kg ,**1000 μg/kg, ***200 μg/kg (http://www.bvl.bund.de/acrylamid/index.htm?pagetitle=Wissensforum+Acrylamid)

However, it is quite clear that for the analysis of acrylamide at <30 μg/kg level, GC-MS after bromination is the best approach. This approach has the advantage of adequate sensitivity with multiple ion confirmation. A further advantage of this technique is that a relatively simple benchtop GC-MS can be employed for acrylamide analysis. Application of GC-MS/MS or coupling to a high resolution MS would even further lower the detection limit of certain foods, approaching the range of 1–2 μg/kg.

To date, there is little evidence that the applied methods have been systematically validated according to international guidelines, such as ISO 17025, although there have been reports of in-house validation. It is also quite obvious that there are significant differences between laboratories in the clean-up procedures applied for both LC and GC methods.

Future Requirements

The analytical laboratories (private, enforcement, food industry) involved in the analysis of acrylamide have, in most cases, developed "in-house" methods, necessitated due to the fact that the analytical methods were not accessible at the time the finding was made public by the Swedish National Food Administration in April 2002. Consequently, laboratories are constantly modifying and adapting their original methods to achieve better method performance regarding LoD, LoQ, analyte recovery, especially in the more "difficult" food matrices. An important conclusion of expert meetings (JIFSAN/NCFST, 2002; Joint European Commission Workshop, 2003) is that no uniform method applicable to all food matrices currently exists, and methods must be individually adapted for each food matrix (sample preparation, extraction, and clean-up procedures). Considering the many different types of foods implicated, this indicates the need for a considerable effort by individual laboratories in method validation, which should follow the ISO 17025 guide and incorporate data on measurement uncertainty.

Other key requirements in the analytical field are the establishment of stringent in-house quality control procedures for all relevant matrices tested. This can be done by inclusion of a standard sample or, if available, reference sample in each analytical series, setting acceptable ranges by using statistical control charts. Furthermore, interlaboratory tests have not been conducted on samples with relatively "low" concentrations of acrylamide. Method reproducibility in the 10–30 μg/kg range has not yet been assessed and should be considered a priority, especially for those foods containing amounts that are a significant part of the staple diet.

Finally, research must also be focused on cheap, rapid screening methods that are reliable and robust. These could then be employed in a quality control environment closer to production in a manufacturing facility or factory environment, for example, in an on-line laboratory, allowing more efficient control and enabling more rapid response if needed.

MECHANISMS OF FORMATION AND MITIGATION

In this section, the results of simple test-tube studies are presented on the precursor molecules, as well as in simple food systems. Hitherto, unpublished evidence of the chemical pathway underlying the formation of acrylamide using model systems is also presented, as well as more applied trial work on potato products, cereals, biscuits, and coffee.

The major mechanistic pathway for the formation of acrylamide in foods so far established is via the Maillard reaction (Becalski et al., 2002; Mottram et al., 2002; Sanders et al., 2002; Stadler et al., 2002; Weisshaar and Gutsche, 2002). The Maillard reaction is, however, a complex desired process, generating a plethora of important flavor and aroma compounds. Thus, any concepts established to minimize the formation of acrylamide must take into account that the organoleptic properties of the cooked foods are not negatively affected (Stadler, 2003). Recent applied work on what one may term a "simple" matrix, such as fried potatoes, illustrates the many variables that could impact the formation of acrylamide. These include the potato cultivar (as well as storage conditions, age, etc.), composition (e.g., reducing sugars, amino acids), and processing conditions (e.g., frying temperature/time regimes, pH adjustment with organic acids, additives) (Biederman et al., 2002b, 2002c; Gertz and Klostermann, 2002; Jung et al., 2003).

This has led to claims of first "solutions" in significantly lowering acrylamide contents in certain foods, which, in the case of fried potato products, entails a combination of measures (Biederman et al., 2002b, 2002c; Grob et al., 2003). However, it must be realized that laboratory or trial work cannot be translated without in-depth studies to a factory production environment. Since acrylamide formation is closely linked to food composition, a number of factors, such as raw material availability (cultivars), potential seasonal variations, fertilization, farming practices, harvesting time, storage, etc., must also be considered. In this context, intensive research will be required for each individual food category, not forgetting the agronomical aspect, which may be a decisive—and long term—factor in some cases, adding to the constraints in finding appropriate and practical solutions.

Fundamental Mechanistic Studies

To date, studies clearly show that the amide amino acid asparagine is mainly responsible for acrylamide formation in cooked foods after condensation with reducing sugars or a carbonyl source. Moreover, the sugar-asparagine adduct, *N*-glycosylasparagine, generated high amounts of acrylamide, suggesting the early Maillard reaction as a major source of acrylamide (Stadler et al., 2002). In addition, decarboxylated asparagine (3-aminopropionamide), when heated, can generate acrylamide in the absence of reducing sugars (Zyzak et al., 2003). Other possible routes involve the Strecker reaction of asparagine, with the Strecker aldehyde as the direct intermediate (Mottram et al., 2002), or a mechanism via acrylic acid (Becalski et al., 2002; Gertz and Klostermann, 2002; Lingnert et al., 2002; Stadler et al. 2003). In fact, a recent report (Yasuhara et al., 2003) on model reaction systems illustrates that under certain conditions, acrolein together with asparagine may generate appreciable amounts of acrylamide, suggesting an important role of acrolein in the formation of acrylamide in lipid rich foods.

Good evidence supporting the early Maillard reaction as a main reaction pathway involving early decarboxylation of the Schiff base, rearrangement to the resulting Amadori product, and subsequent β-elimination to release acrylamide, has been presented (Yaylayan et al., 2003). Experimental results, which, in essence, corroborate this route are presented, as well as other potential (minor) pathways to acrylamide.

Labelling Experiments

It has been shown, by labelling experiments, that the carbon skeleton of acrylamide stems from asparagine (Figure 4). Condensation of asparagine with $^{13}C_6$-glucose resulted in unlabeled acrylamide, indicating that sugar carbon atoms are not used to construct the acrylamide molecule. On the contrary, copyrolysis of $^{15}N_1$-amide-labelled asparagine and glucose led to incorporation of the N-15 label in acrylamide. However, no isotopic enrichment of acrylamide was observed after the same reaction with $^{15}N_1$-α-amino-labelled asparagine and glucose (Stadler et al., 2002). These results indicate that the entire carbon skeleton of acrylamide originates from asparagine. Reducing sugars promotes this reaction, which formally represents a decarboxylation and deamination step accompanied with double bond formation via β-elimination.

Pyrolysis of Model Analogue Compound

The initial phase of the Maillard reaction leads to the formation of *N*-glycosyl derivatives of the amino acids. For example, glucose and asparagine give rise to *N*-(D-glucos-1-yl)-L-asparagine I, which is in equilibrium with the Schiff base II, as shown in Figure 5 (step A). Usually and preferably in aqueous

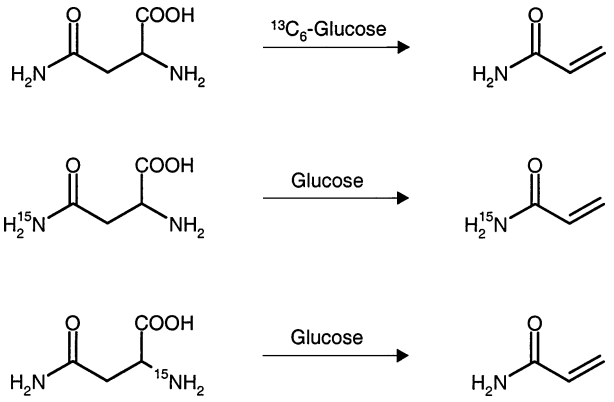


Figure 4 Labelling experiments using 13-C labelled sugar or 15-N labelled asparagine.

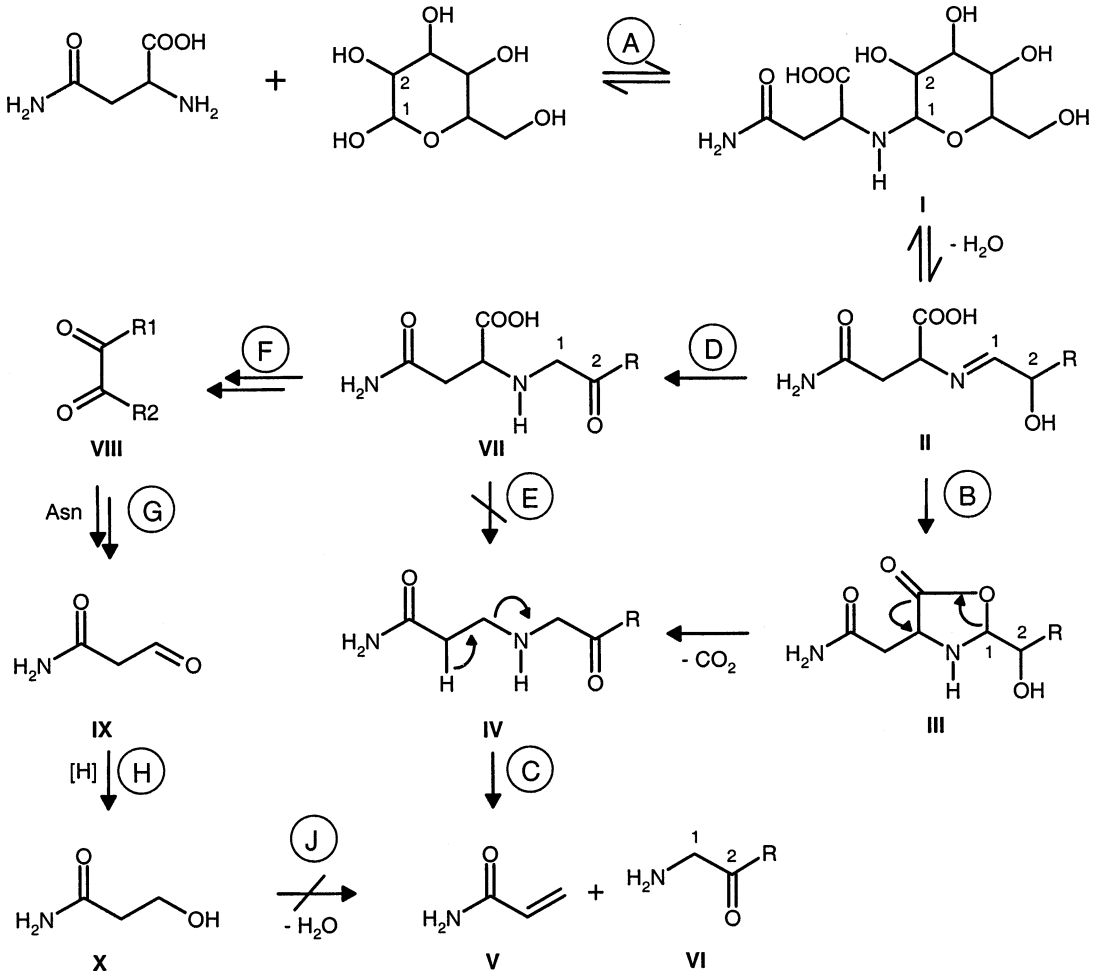


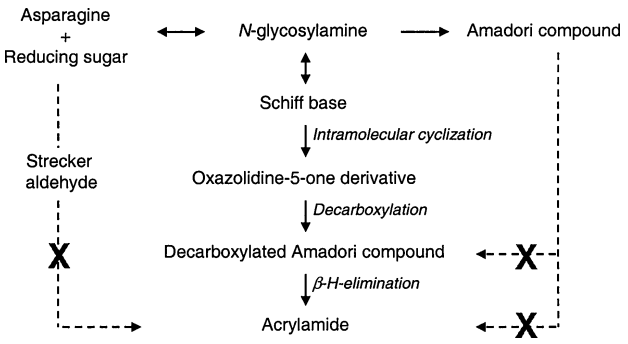
Figure 5 Proposed mechanisms of acrylamide formation in glucose/asparagine copyrolysates through the Maillard reaction.

systems, this reaction will proceed via pathway D to furnish 1-amino-1-deoxyketoses of amino acids, known as Amadori compounds (VII), which represent the first stable intermediates generated in aqueous systems, as a result of the early Maillard reaction cascade. In low moisture systems, however, *N*-glycosyl compounds are the first stable intermediates, which can be isolated in their salt form. In the glucose/asparagine system, the *N*-glucosylasparagine (I in Figure 5) will lead to the Schiff base II that is relatively stable under these dry conditions. The Schiff base can undergo intramolecular cyclization (step B) and the resulting oxazolidine-5-one derivative III decarboxylate, leading to the corresponding Amadori compound IV. The decarboxylated Amadori compound can decompose via β-elimination (step C) resulting finally in acrylamide V and an amino sugar VI.

The pathway of acrylamide formation in a glucose/asparagine system seems to occur prior to the Amadori rearrangement (step D). Indeed, the acrylamide amount found from *N*-glucosyl asparagine I is 10 times higher than from the Amadori compound VII, thus, suggesting that the direct decarboxylation of the Amadori compound VII to IV is not favored under these conditions. Furthermore, protection of the sugar ring and the acidic function does not affect the acrylamide formation. It appears that as soon as the Amadori product is formed, it will react to be degraded through the classical Maillard reaction pathways leading to 1- and 3-deoxyosones and, thus, favoring the formation of flavor and color compounds, instead of acrylamide (Scheme 2).

Detection of the Intermediate Beta-Alanine Amide by LC-MS

During the typical heating of foods, reducing sugars react with amino acids, initiating a cascade of events that lead to the



Scheme 2 Main pathway leading to acrylamide in food.

browning of foods, known as the Maillard reaction. This process is known to generate more reactive monocarbonyl and dicarbonyl compounds that are proposed to be responsible for the browning reaction. To better understand the mechanism of acrylamide formation from asparagine, Zyzak et al. (2003) investigated the ability of other carbonyl containing compounds to generate acrylamide in the potato snack model system. They found that a variety of carbonyl sources could generate acrylamide from asparagine under heat, including glucose, 2-deoxyglucose, ribose, glyceraldehydes, glyoxal, and decanal. Current understanding is that the first step in acrylamide formation is the Schiff base formation between the carbonyl and the α-amine of asparagine. In one of their experiments, they used 2-deoxyglucose as the carbonyl source and showed that it also formed comparable amounts of acrylamide. Since 2-deoxyglucose does not have a hydroxyl group adjacent to the carbonyl, it can only form the Schiff base adduct and cannot undergo the Amadori rearrangement, which leads to the formation of dicarbonyl compounds, e.g., 3-deoxyglucosone. Combined, these observations suggest the necessity of carbonyls in the formation of acrylamide, and that dicarbonyls are not required for the formation of acrylamide from asparagine.

Based upon the isotope-labeling experiments and carbonyl studies (Zyzak et al., 2003), the mechanism illustrated in Figure 6 is proposed. The α-amine group of free asparagine reacts with a carbonyl source, forming a Schiff base. Under heat,

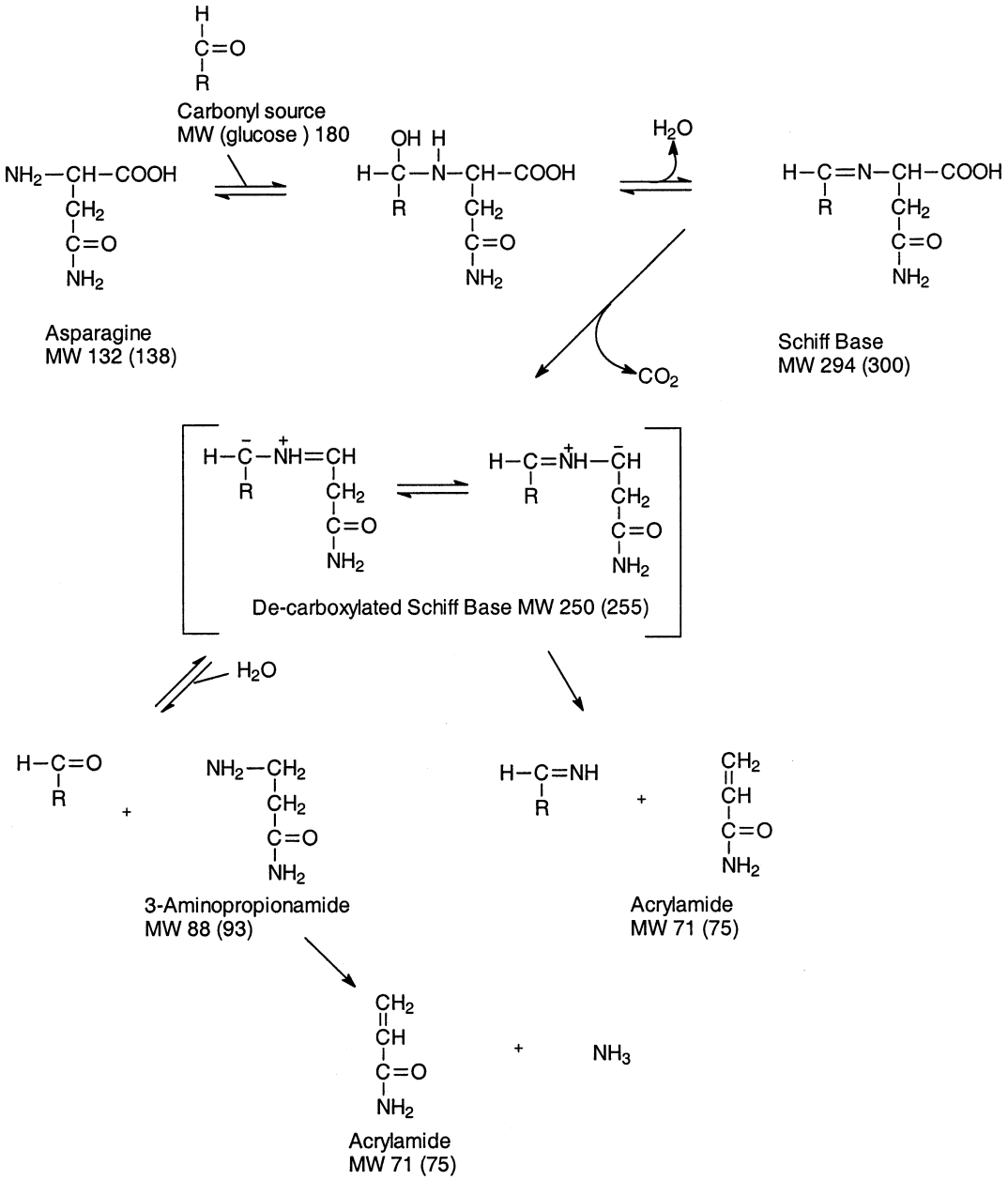


Figure 6 Proposed mechanism of acrylamide formation from carbonyl/asparagine with confirmed intermediates of Shiff-base, de-carboxylated Shiff-base and 3-aminopropionamide.

the Schiff base de carboxylates (facilitated by delocalization of negative charge that Schiff base formation allows), forming a product that can react one of two ways. It can hydrolyze to form 3-aminopropionamide, which can further degrade via the elimination of ammonia to form acrylamide when heated. Alternatively, the decarboxylated Schiff base can decompose directly to form acrylamide via elimination of an imine. Additional evidence to support this mechanism is developed using an aqueous reaction system comprising dextrose and asparagine. These intermediates were confirmed in model studies (Zyzak et al., 2003). These mechanisms (Figures 5 and 6) appear to be the most direct and realistic pathways of acrylamide formation from asparagine and a reactive carbonyl.

Pyrolysis of 3-Hydroxy-Propanamide and Temperature/Time Kinetics of Acrylamide Formation

An alternative pathway (Mottram et al., 2002) to consider is via the Strecker reaction of asparagine (step G) in the presence of dicarbonyls VIII, generated from the Amadori compound VII (step F) and leading to the Strecker aldehyde IX (Figure 5). Reduction of IX to the corresponding alcohol X and its subsequent dehydration (step J) could release acrylamide. Thus, the "Strecker alcohol" of asparagine (X) may represent a direct precursor of acrylamide after a thermally induced loss of water. However, upon pyrolysis of the pure compound in a closed system, only relatively low amounts of acrylamide were generated up to about 180°C (Figure 7), and the majority of the starting material could be recovered after the reaction as determined by an LC-MS assay. Thus, on the basis of these model experiments, the Strecker pathway can be considered minor in the formation of acrylamide from asparagine and reducing sugars.

Intermediacy of Acrylic Acid in the Formation of Acrylamide

In theory, acrylic acid could furnish acrylamide by an amino dehydroxylation reaction, which essentially requires ammonia (March, 2001). Acrylic acid can be procured from acrolein, which can be formed by a multi-step process from the thermal degradation of lipids/glycerol (Umano and Shibamoto, 1987), carbohydrates (Heyns et al., 1966), amino acids (Alarcon, 1976), or Maillard products (Ferretti and Flanagan, 1971). Another potential pathway to acrylic acid could be the Maillard reaction, either from aspartic acid analogous to the route described for acrylamide (Yaylayan et al., 2003), or Strecker degradation products involving 2-propenal (Vattem and Shetty, 2003). In the case of the former, the key reactants would be free aspartic acid, which provides the backbone of the acrylic acid molecule and a carbonyl source.

Preliminary studies involving the thermal condensation of equimolar amounts of glucose with aspartic acid and subsequent isotope dilution LC-MS/MS analysis indeed confirmed that acrylic acid is formed at concentrations comparable to that of acrylamide under similar conditions (reactant concentration, time, temperature) (Stadler et al., 2003). As illustrated in Figure 8, acrylic acid reaches >5 mmol/mol amino acid and >14 mmol/mol at 230°C and after 5 m in glucose-aspartic acid and fructose-aspartic acid mixtures, respectively. Mild pyrolysis of the amino acid alone did not yield acrylic acid.

Supplementation of a fructose-aspartic acid reaction mix with additional amino acids known to deamidate at relatively low temperatures (110°C), such as glutamine (Sohn and Ho, 1995), could provide the ammonia source to drive the reaction to acrylamide. Indeed, the addition of glutamine (2-fold molar excess vs aspartic acid) to fructose-aspartic acid mixtures reached approx. 5% efficiency of acrylamide formation versus the asparagine route (data not shown).

Therefore, based on the results obtained in the model systems, both acrylic acid and acrylamide could potentially be formed in foods with an abundance of free aspartic acid and the availability of sugars, ammonia being generated by the decomposition of amino acids present either in the free or bound form. Acrylic acid has never been considered in food as a processing-derived compound, and thus most of the data available in the literature is related to (poly)acrylates used in food packaging materials (EC Document, 2002).

However, at this stage, it is not possible to predict whether the acrylic acid pathway is of only marginal importance, or if

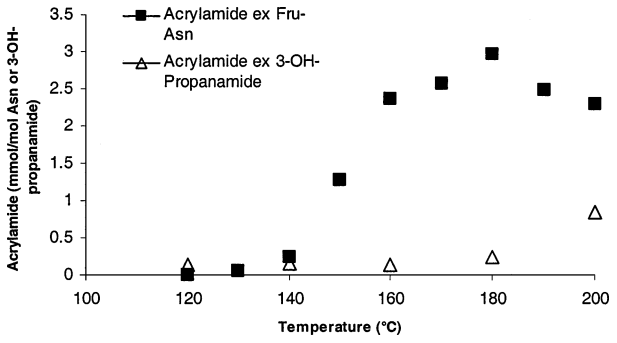


Figure 7 Formation of acrylamide from 3-hydroxy-propanamide and fructose-asparagine mixtures under the same experimental conditions over a temperature range 120–200°C (pyrolysis time 60 m, closed systems). All entries are averages of two independent determinations.

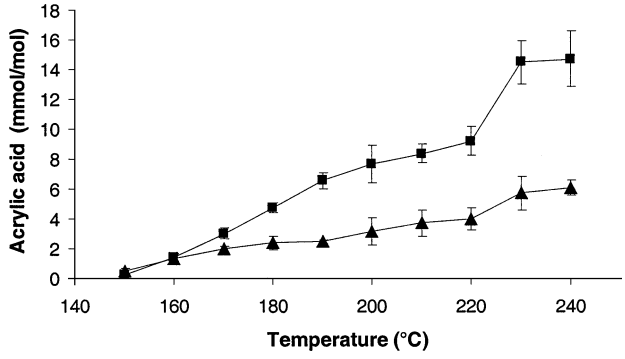


Figure 8 Formation of acrylic acid in fructose-aspartic acid (-■-) and glucose-aspartic acid (-▲-) mixtures as a function of temperature (pyrolysis time 5 m, closed systems). All entries are averages of four independent determinations.

it may be a favored mechanism in any cooked food. Inspection of different foods known to release acrylamide, but with low concentrations of free asparagine, may provide a clearer picture. Furthermore, acrylic acid is freed at relatively high temperatures (optimum >220°C), suggesting that thermal energy barriers may be greater for the reaction to proceed.

Model Studies with Food Matrices

A few studies have been reported (Amrein et al., 2003; Becalski et al., 2003; Biedermann et al., 2002b, 2002c) using food models that usually follow on the fundamental test tube experiments with pure compounds. Model studies using food matrices mimic more closely the conditions of home cooking or industrial preparation, and therefore, contribute to designing models that may identify the most salient factors that determine acrylamide formation in the different food categories.

Several different foods were assessed for their impact as a matrix, in the model system. For each matrix, the acrylamide formation was measured before and after washing to determine the following: (a) to what extent they contained reactive constituents (precursors, such as reducing sugars, free asparagine); (b) if the constituents could be removed. The washing step was performed by suspending the material in water, centrifugation, removal of the supernatant, and re-suspension in water. This procedure was repeated in one or more cycles (usually two cycles).

The food matrices studied were potato, rye flour, whole wheat flour, potato starch, corn starch, and beef. The potato and beef samples were first homogenized in water before they were transferred to the test tubes, while the flours and starches were used without prior treatment, suspended in water to form a slurry, and subsequently, transferred to the test tubes.

As shown in Table 2, the potato, and to a lesser extent, the wheat and rye flour, produced some acrylamide upon heating. This probably reflects, to a major extent, the amount of free asparagine in the raw agricultural commodities (See page 337: *Processing Trials—Potatoes—Impact of Raw Material Variability*), explaining the relatively higher amounts detected in potato.

Table 3 Acrylamide formation* from reaction between asparagine (0.75 mmol) and various sugars (0.75 mmol) in a washed whole wheat flour matrix

Sugar	Acrylamide (μg)**
Glucose	72
Fructose	110
Sucrose	92

*1 g, washed 2 cycles; heated at 200°C for 30 minutes.
**Entries are expressed as acrylamide per reaction vessel (1 g starting material); averages of 2 independent determinations.

The washing step had a clear effect in lowering the acrylamide formation in potato, wheat, and rye, corroborating the importance of key reactants, such as free asparagine. Corn and potato starch, which are expected to have no free asparagine, did not contribute significantly to the formation of acrylamide. Both were commercial starches (corn starch Maizena from Bestfood Nordic AB, <0.5% protein according to the producer; potato starch Potatismjöl from Lyckeby Stärkelsen, <0.5% protein according to the producer).

Impact of Carbohydrates

The influence of the sugar component on the acrylamide formation from asparagine was studied in experiments with a washed whole wheat flour matrix. The sugars glucose, fructose, and sucrose were compared at equimolar amounts relative to asparagine. As illustrated in Table 3, all three sugars, together with asparagine, formed acrylamide—even sucrose, in spite of the fact that it is not a reducing sugar. Fructose tended to be somewhat more effective than glucose. The reaction with sucrose resulted in acrylamide amounts similar to those recorded for fructose and glucose. A plausible explanation to acrylamide formation from sucrose is that the sucrose is hydrolyzed upon thermal treatment to the individual monosaccharides. It considered that one sucrose molecule could then, in theory, provide two reducing hexoses, i.e., a molar ratio of sugar to amino acid of 2:1. Biedermann et al. (2002c) also reported fructose to be more efficient than glucose in forming acrylamide (in a

Table 2 Acrylamide formation in various matrices* before and after washing

Matrix	Free asparagine content in the raw agricultural commodity (mg/100 g FW or DW) (Typical Literature values)	Reference for free Asparagine Content	Acrylamide (μg)**	
			Unwashed	Washed
Potato homogenate	87–211 FW	Mack and Schjoerring, 2002; Brierley et al., 1997	3.1	0.2
Whole wheat flour	8.2 DW	Benedito de Barber et al., 1989	0.3	0.1
Rye flour	20–284 DW	Dembinski and Bany, 1991	0.8	0.2
Corn starch	59–107 DW	Wang et al., 2001	0.01	0.01
Potato starch	87–211 FW	Mack and Schjoerring, 2002; Brierley et al., 1997	Nd	<0.1
Beef homogenate	—		0.1	—

*1 g of the matrix was heated at 200°C for 30 minutes.
**Entries are expressed as acrylamide amount per reaction vessel (1 g starting material).
FW = fresh weight; DW = dry weight; Nd = not determined.

potato model). Similarly, Pollien et al. (2003) found fructose to be more efficient in generating acrylamide from asparagine, compared to glucose. Comparisons between various sugars were also reported by Stadler et al. (2002). They found fructose, galactose, lactose, and sucrose to release acrylamide with comparable yields. Yaylayan et al. (2003) performed model studies at high temperature (350°C) and found sucrose to be approximately three times as potent as glucose or fructose alone in forming acrylamide with asparagine.

This concludes that acrylamide is formed in comparable amounts with several mono- or disaccharides. Even non-reducing sugars, such as sucrose, are efficient reactants leading, after thermally-induced hydrolysis, to the release of reducing sugars that are then available to react with the α-NH_2 group of asparagine via the Maillard route.

Impact of Lipids and Vegetable Oils

The possible role of lipids in acrylamide formation has been intensively debated. In particular, the influence of the frying oil in the frying processes. One hypothetical mechanism that has been suggested for the acrylamide formation is via acrolein, formed from the degradation of lipids (oxidized fatty acids or glycerol). Lipids heated at high temperature can lead to the formation of acrolein (Umano and Shibamoto, 1987). Acrolein can further react by oxidation to furnish acrylic acid or by formation of an intermediate acrylic radical. Both species could then—in the presence of a nitrogen source and under favoreable reaction conditions—provide acrylamide (Yasuhara et al., 2003). Becalski et al. (2003) reported a certain impact due to the choice of oil when frying potato slices. In limited experiments, the use of both paraffin oil and olive oil resulted in somewhat higher acrylamide contents than frying in corn oil. Tareke (2003) also reported increased acrylamide formation when adding oil during the heating of potato. The contents of acrylamide formed were highest in the potatoes heated with the addition of sesame oil (compared to corn oil and olive oil). Biedermann and colleagues (2002c) reported no significant effect of the addition of edible oil, or glycerol to a dry potato model prior to heat treatment. Practical potato frying experiments have not indicated any influence of oil quality. Frying in abused oil did not influence the acrylamide formation.

Additions of glycerol, corn oil, and olive oil, were also tested in a model system. Potato homogenate (unwashed) was used as the matrix, and the experiments were conducted both with and without added asparagines and glucose. As shown in Table 4, the addition of vegetable oils or glycerol did not have a significant impact on the formation of acrylamide in this model. Therefore, oils or glycerol do not contribute to the formation of acrylamide via intermediates, such as acrolein /acrylic acid, as recently proposed (Yasuhara et al., 2003).

A selection of twelve commercially available vegetable oils was exposed to temperatures typically encountered under processing conditions, such as frying and acrylamide concentrations measured in the oils after a defined heating period. Two

Table 4 Impact of lipids on acrylamide formation*

	Acrylamide (μg)**	
Lipid addition	Without asparagine-glucose	With asparagine-glucose
No addition	3.1	30
Glycerol (2%)	4.3	22
Corn oil (2%)	3.9	38
Olive oil (2%)	4.9	31

*Matrix: Potato homogenate (unwashed) with or without added asparagine (0.5 mmol)–glucose (0.5 mmol); heated at 200°C for 30 minutes.
**Entries are expressed as acrylamide per reaction vessel (1 g starting material); averages of 2 independent determinations.

of the vegetable oils chosen did indeed release acrylamide after thermal treatment, namely a soya bean and an "organic" wheat germ oil. Acrylamide formation was significantly higher in the latter, increasing near linearly up to 135°C after a 1 h heating period (Figure 9). This commercial oil is a product labelled "biological," and it is classified as an unrefined, cold pressed oil, which may contain relatively higher amounts of proteinaceous material and carbohydrates. To potentially identify the nitrogen source in the oils, proteins were extracted following cold precipitation with acetone (Hidalgo et al., 2001), with subsequent SDS-PAGE and silver staining of the gel. No clear correlation of protein content and acrylamide formation could be established, since protein bands migrating at similar MW (estimated 67 KDa) were also distinguishable in vegetable oils that did not lead to the formation of acrylamide after thermal treatment (qualitative comparison by addition of fixed amounts of protein standards).

It is difficult to draw any firm conclusions from these limited tests, but the lipids studied appeared to have only a marginal influence on the acrylamide formation. Thus, so far, there is no conclusive evidence that lipids have a significant impact on acrylamide formation.

Impact of Proteins

It has been reported that amounts of acrylamide are relatively lower in meat products (Becalski et al., 2003; Tareke et al., 2002). There are various possible explanations of the findings of

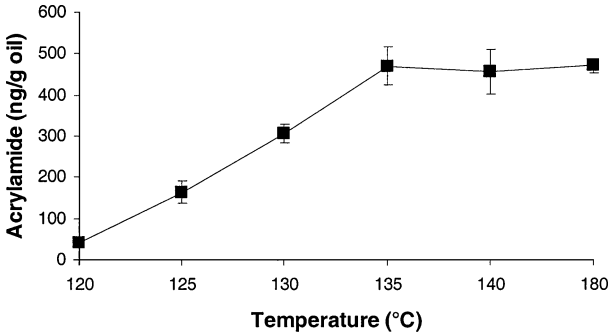


Figure 9 Impact of temperature on the formation of acrylamide in a commercially available vegetable oil (cold-pressed wheat germ), heating time 10 m. All entries are averages of three independent determinations.

Table 5 Acrylamide formation* from asparagine—glucose in different matrices

Matrix	Glucose—Asparagine addition	Acrylamide (μg)**
Beef homogenate	none	0.1
Beef homogenate	0,25 mmol glucose + 0,25 mmol asparagine	14
Beef homogenate	0,5 mmol glucose + 0,5 mmol asparagine	6
Beef homogenate (washed)	0,5 mmol glucose + 0,5 mmol asparagine	12
Potato homogenate (washed)	0,5 mmol glucose + 0,5 mmol asparagine	30
Whole wheat flour (washed)	0,5 mmol glucose + 0,5 mmol asparagine	62
β-lactoglobulin	0,25 mmol glucose + 0,25 mmol asparagine	3.5

*Heat treatment: 200°C for 30 minutes.
**Entries are expressed as acrylamide per reaction vessel (1 g starting material); averages of 2 independent determinations.

lower acrylamide concentrations in cooked beef, and major reasons may be that beef contains relatively little free asparagine (Feidt et al., 1996) and represents a "high-moisture" system. Moreover, constituents in beef may inhibit the reaction or favor competitive reactions. Alternatively, the acrylamide formed might be "bound" by the matrix or react chemically with constituents (inherent nucleophiles) in the matrix. The results of experiments with a meat (beef) matrix are summarized in Table 5. The addition of beef to glucose-asparagine mixtures reduces the measured acrylamide amount to approximately 5–6 fold. Biedermann and colleagues (2002c) reported that the degradation of acrylamide was more pronounced in a beef system than in other matrices, indicating an elimination mechanism. The binding of acrylamide to proteins/amino acids, in the beef matrix, which is rich in SH-containing amino acids, such as cysteine, is one plausible explanation. Tareke (2003) also showed that mixing minced cod fish into potato patties prior to heating significantly lowered the amounts of acrylamide. The acrylamide released was inversely proportional to the amount of cod. The results of the β-lactoglobulin experiment illustrated in Table 5 supports the idea that proteins may indeed play an important role in lowering acrylamide contents in food.

Experiments were also performed reacting glucose with combinations of asparagine and other amino acids. The addition of cysteine to the glucose—asparagine system decreased the formation of acrylamide (Table 6). Again, the explanation could be either that the cysteine influences the reaction paths leading to acrylamide, or that acrylamide is reacting with cysteine. Becalski et al. (2003) reported that the addition of lysine to a glucose—asparagine model system lowered the yield of acrylamide. The same tendency is shown in Table 6 for lysine, even if less pronounced than cysteine.

Table 6 Influence of lysine and cysteine on acrylamide formation in a potato starch matrix with addition of asparagine—glucose*

Amino acids**	Acrylamide (μg) ± SD***
Control (only potato starch)	<0,1
Asparagine	46 ± 1
Asparagine (1 mmol)	50 ± 16
Asparagine + Lysine	34 ± 4
Asparagine + Cysteine	10 ± 4

*Matrix: Potato starch (washed); all 1 mmol glucose; heat treatment: 200°C for 30 minutes.
**Each 0.5 mmol unless otherwise stated.
***Entries are expressed as acrylamide per reaction vessel; averages of 3 independent determinations.

Impact of Antioxidants

Related to discussions of the impact of vegetable oils is the possible influence of antioxidants. Tareke (2003) found that the addition of antioxidants (BHT, sesamol, Vitamin E) to meat prior to heating enhanced the formation of acrylamide, probably by protection of acrylamide against free radical initiated reactions. It is also suggested that the increased acrylamide formation, when adding oil during the heat treatment of potato, might be due to the antioxidants in the oils, differences observed between oils was related to their antioxidant status.

On the other hand, Becalski et al. (2002) reported decreased acrylamide formation when adding rosemary herb to the oil used for frying potato slices. Rosemary is known for its antioxidant content, but this effect could, of course, be due to many other factors. Relatively lower amounts of acrylamide after the addition of a flavonoid spice mix have also been reported by Fernández et al. (2003). The spice mix (liquid) was added to potato slices before frying, and a powder spice mix was also added to the potato slices after frying. After a 4 day incubation time, the acrylamide contents were reported to be reduced by up to 50% in the spice mix treatment. Biedermann et al. 2002c reported a weak decrease of the acrylamide formation by the addition of ascorbic acid to a potato model. The frying of beef under argon atmosphere (to protect against possible oxidation) had no significant effect on the acrylamide formation (Tareke, 2003).

Based on the current evidence, it is difficult to draw firm conclusions on either the positive or negative impact of antioxidants. The few reports published appear conflicting. Retarding effects have been demonstrated with flavonoids (or spice extracts). This may not necessarily be due to the antioxidative properties of those additives.

Impact of Additives—Ammonium Carbonate

Industrial baking trials indicate that acrylamide formation is increased when ammonium carbonate is used as a baking aid

(unpublished data). The possibility that ammonium carbonate could provide an extra source of nitrogen cannot be ruled out. When adding ammonium carbonate to a glucose–asparagine system (without food matrix), no increased acrylamide formation was observed. Since the addition of ammonium carbonate will influence the pH of the system and promote the formation of a Schiff base, a control was also included, where pH was adjusted back to the original pH. Ammonium carbonate was also added to a system of glucose and an amino acid mixture of serine, threonine, alanine, proline and phenylalanine, to determine whether ammonium carbonate might produce acrylamide with other amino acids (release of ammonia). The results of these model experiments showed no clear impact of ammonium carbonate on acrylamide formation. Further studies were done in wheat flour matrices. In the case of unwashed whole wheat flour as a matrix, an increase in the amount of acrylamide formed was observed when ammonium carbonate was added. However, at this stage no firm conclusions can be drawn, and more extensive tests must be conducted.

Impact of Enzymes Asparaginase and Amidase

Studies indicate that the side chain amide group of asparagine is incorporated into the amide bond of acrylamide. A logical confirmation of this mechanism would be to degrade this amide bond through hydrolysis, with either acid or enzyme, and to measure acrylamide in a food. A way to accomplish this specifically for asparagine is to use the enzyme asparaginase, which catalyzes the hydrolysis of asparagine into aspartic acid and ammonia. Zyzak et al. (2003) evaluated the effectiveness of asparaginase to reduce acrylamide formation in a mashed potato product heated in a microwave oven. Asparaginase pretreatment of the snack produced an asparagine reduction of 88% and an acrylamide reduction of greater than 99%, when compared to samples prepared by the exact same process without the enzyme. This result, again, shows that the major route to acrylamide formation, in a potato product, is through asparagine. Such possible measures require careful assessment in terms of their practical feasibility under actual food production conditions, and the consequences such treatments may have for the quality and safety of the final product.

Processing Trials

Potatoes

Fried potatoes are in the food category with probably the highest concentrations of acrylamide recorded so far (Friedman, 2003), and in which the most work has been done to control or reduce acrylamide. Numerous possible avenues of reduction of acrylamide in potato products, particularly french fries, have been highlighted in several recent reports (Biedermann et al., 2002b, 2002c; Grob et al., 2003; Haase et al., 2003, Jung et al., 2003; Noti et al., 2003). These entail controlling the temperature of storage of the raw potato (Grob et al., 2003; Noti et al., 2003), selection of certain varieties (Biedermann et al., 2002c; Haase et al., 2003), modifying processing (frying) conditions (Biedermann et al., 2002b, 2002c; Grob et al., 2003; Haase et al., 2003, Jung et al., 2003), and assessing the impact of protein coating (Vattem and Shetty, 2003). Any modifications performed on the raw material constituents will inevitably impact the Maillard reaction and its products and, concomitantly, the organoleptic properties (taste and color) of the cooked food. However, even though small scale and laboratory trials have shown that products such as french fries can be prepared with acrylamide amounts below 100 μg/kg (Grob et al., 2003), all these measures must be placed in the perspective of consumer acceptance, not forgetting those related to the supply chain management and logistics of harvesting, storage, and transport of the raw potatoes. The following sub sections highlight the variability in potato composition, as well the effect of different frying processes and sugars on acrylamide formation.

Impact of Raw Material Variability. The experimental trials that have been conducted so far on acrylamide in potatoes have shown that the major determinants of acrylamide formation are reducing sugars (mainly glucose and fructose), as well as the (free) amino acid asparagine. The content of sugar (glucose/fructose) in the raw potato is well correlated ($r^2 = 0.85$) to the amount of acrylamide formed upon heating (Biedermann et al., 2002c). A wide range of potatoes were analyzed for free amino acids and sugars (glucose, fructose, and sucrose). As already documented in several reports, among the various free amino acids measured (Figure 10), the content of free asparagine was the highest. Widely varying concentrations of asparagine, glucose, fructose, and sucrose were observed (Table 7), as also shown recently by Amrein et al., (2003).

This variability may be one important explanation for the difference in the amounts of acrylamide that may be formed in the products during processing. The reasons for this large spread in asparagine and reducing sugars is probably due to multiple factors, such as potato cultivar, farming systems, fertilization, pesticide/herbicide application, time of harvest, storage time, and temperature. In fact, fertilization has been shown to considerably impact free amino acid concentrations (Eppendorfer, 1996). This finding could not be confirmed by Amrein et al. (2003), and the authors emphasize in their work the importance of cultivar selection in controlling acrylamide content. Clearly, intensive investigations will need to be conducted in the future to enable a better understanding of how these many factors may affect the raw material composition and their variability.

Table 7 Typical contents of free asparagine, glucose, fructose, and sucrose in raw potatoes

Number of samples	Constituent	As %age dry wt of potato
226	Asparagine	0.23–3.94
139	Glucose	0.02–2.71
139	Fructose	0.02–2.5
139	Sucrose	0.14–4.23

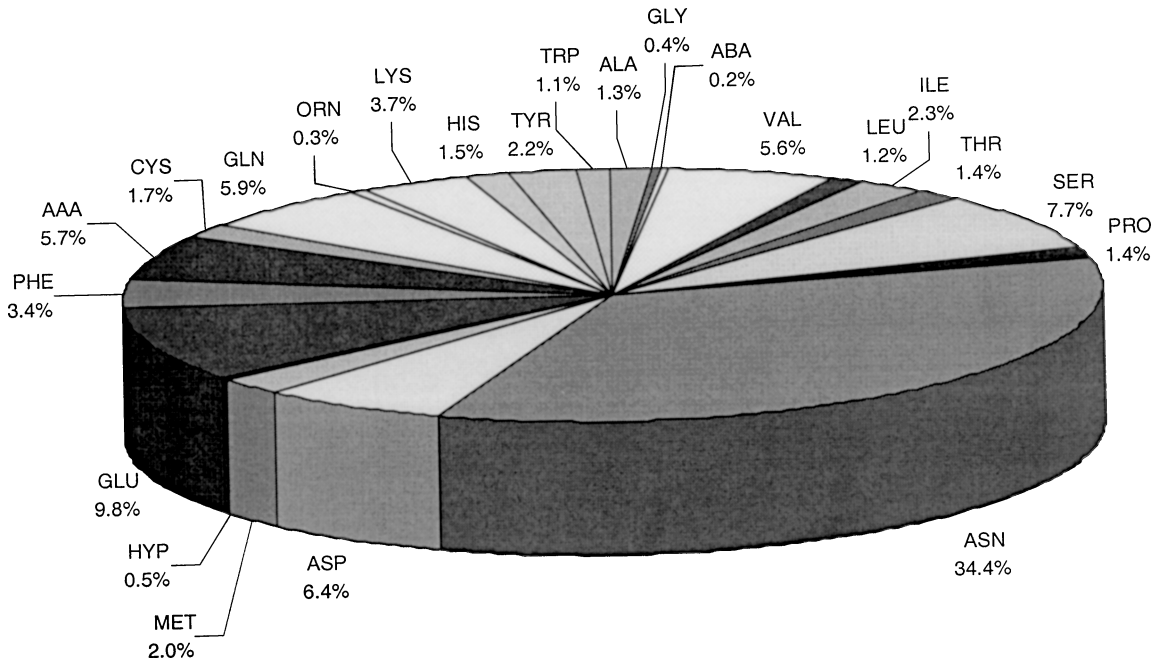


Figure 10 Distribution of free amino acids (wt% based on total free amino acids) in potato (average of 124 independent samples). AAA—amino adipic acid, ABA—amino butyric acid, ALA—alanine, ASN—asparagine, ASP—aspartic acid, CYS—cysteine, GLN—glutamine, GLU—glutamic acid, GLY—glycine, HIS—histidine, HYP—hydroxyproline, ILE—isoleucine, LEU—leucine, LYS—lysine, MET—methionine, ORN—ornithine, PHE—phenylalanine, PRO—proline, SER—serine, THR—threonine, TRP—tryptophan, TYR—tyrosine, VAL—valine. Arginine was not quantified by the method.

Effect of Par Frying on Acrylamide Formation. Acrylamide content was strongly related to the reconstitution temperature of the finished product (Figure 11) and not related to the par frying temperature. The reconstitution temperature refers to the temperature at which the frozen par frying material is finished frying. After reconstitution, the product has the desired texture for consumption. Par frying a cut size of 3/8 lab produced french fries (reference to the dimensions of the raw potato strip, i.e., the size of the strip is 3/8 of an inch or 9.5 mm^2) at various temperatures had little effect on the acrylamide level after reconstitution at 160°C and 180°C. At 180°C, however, more variation was seen across the temperature range. In general, altering the par frying process did not generate substantial reductions in acrylamide.

Effect of Glucose Addition During Processing. Glucose content has a positive and significant effect on acrylamide formation in the finished product (Becalski et al., 2003; Biedermann et al., 2002b; Haase et al., 2003). French fries dipped in a glucose solution before par frying showed a concentration-dependent effect on acrylamide formation (Figure 12). The acrylamide content increased with reconstitution temperatures at all glucose

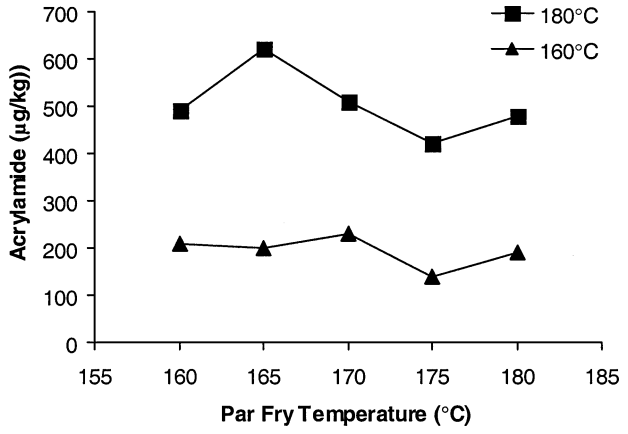


Figure 11 Acrylamide content of reconstituted lab scale processed french fries par fried at various temperatures to constant weight loss.

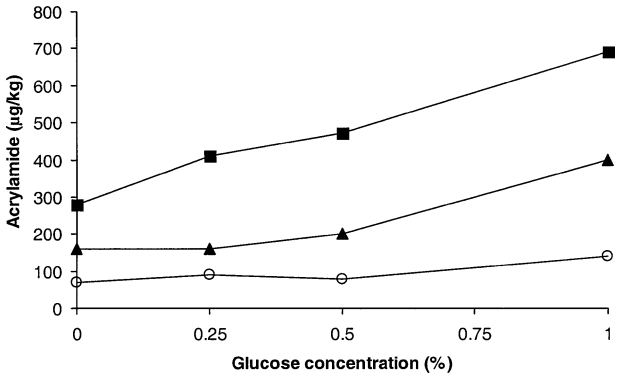


Figure 12 Changes in acrylamide content of lab-scaled produced 3/8 french fries as a function of glucose dip concentration and reconstitution temperature (-■- 180°C, -▲- 160°C, -○- 140°C).

concentrations. At 140°C, the effect of glucose was minimal, whereas sugar concentration appeared to play a greater role in the formation of acrylamide when reconstituted at 180°C. At glucose contents above 0.5%, acrylamide concentrations increased more substantially.

Final Deep-Fat Frying: Reconstitution from Frozen

Previous work (effect of sugar and par frying) has shown that the formation of acrylamide occurs primarily during final frying. In a detailed examination of this observation, results showed acrylamide formation followed a non-linear rate of formation with increased temperature (Figure 13).

At high temperatures, well above the normal frying range, the negative affect of overcooking (i.e., acrylamide formation) becomes more pronounced. At low frying temperatures and longer frying times, acrylamide was substantially reduced. However, negative quality issues, such as higher fat uptake and poorer texture, become more apparent.

Cereals

Breakfast cereals are made by a variety of distinct processes that yield many forms of flake, puffs, extrudates, and biscuit-like pieces. Common to all of the processes is that grains are cooked in water, in most cases, with added sugars (even if only malt as flavoring) to a mass that can be formed into individual cereal pieces. The individual cereal pieces are then dried in air, generally in some form of oven that develops toasted flavors. The Maillard reaction develops flavors and color in both the cooking and the toasting steps of the process. Results to date show that most or all of the acrylamide present in cereals is formed in the toasting step.

The dominant feature of the acrylamide content of cereal is the wide range of values shown between batches of the same cereal processed under the same conditions and apparently the same product (Table 1). This variability creates a difficulty for experimental design—a doubling or halving of acrylamide content can happen simply between experiments. To date, no modification to process has had a beneficial effect as large as this variation. An understanding of what is driving this variation is needed.

Some systems were studied in which wheat was cooked with a variety of recipes and processes. They allow a vivid illustration of the complexity that arises when making the transition from the chemistry of the Maillard reaction in solution to that in food. Figure 14 is a thermal image of an all wheat breakfast cereal biscuit removed from a baking oven and immediately broken open in front of the thermal imaging camera. The oven temperature was 220°C, but the cooling effect of water evaporating from the biscuit means that the biscuit surface never exceeds 120°C, and the core of this biscuit never exceeds 80°C. This is an extreme case of the general situation that for products toasted in air, there is no simple relation of product temperature to toaster temperature.

Some reports in the literature would lead one to expect little or no acrylamide to form at the low temperature of the biscuit

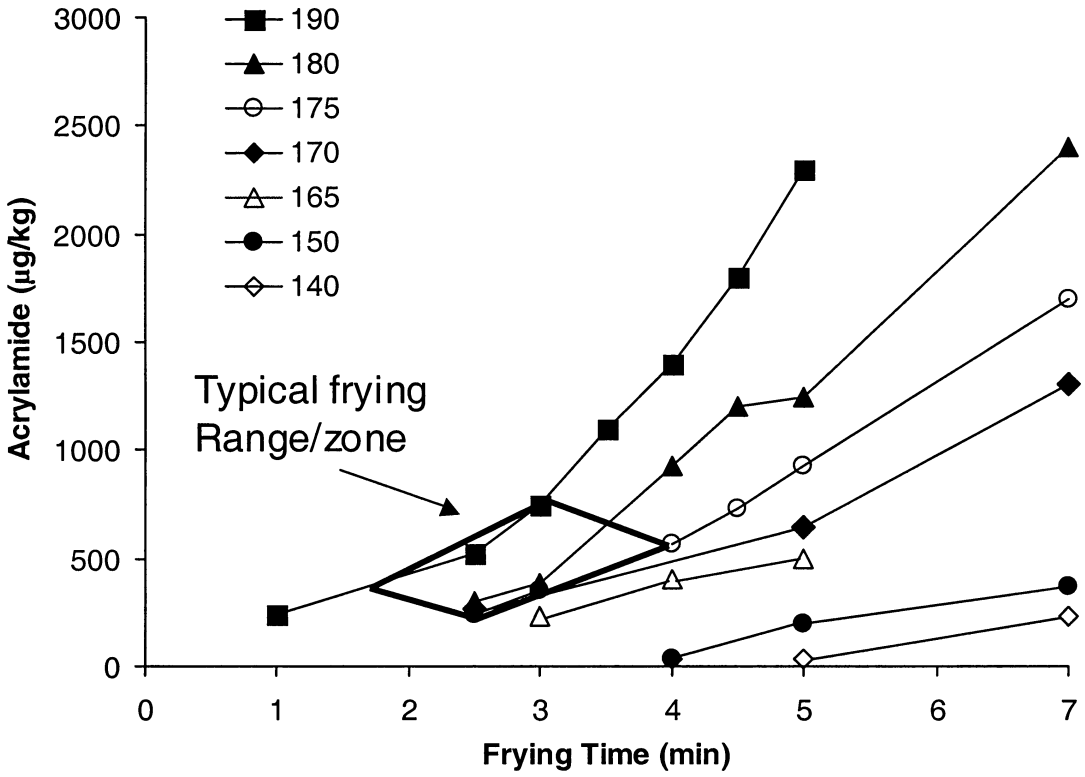


Figure 13 Influence of reconstitution temperature (°C) and frying time on acrylamide formation in 3/8 lab processed french fries.

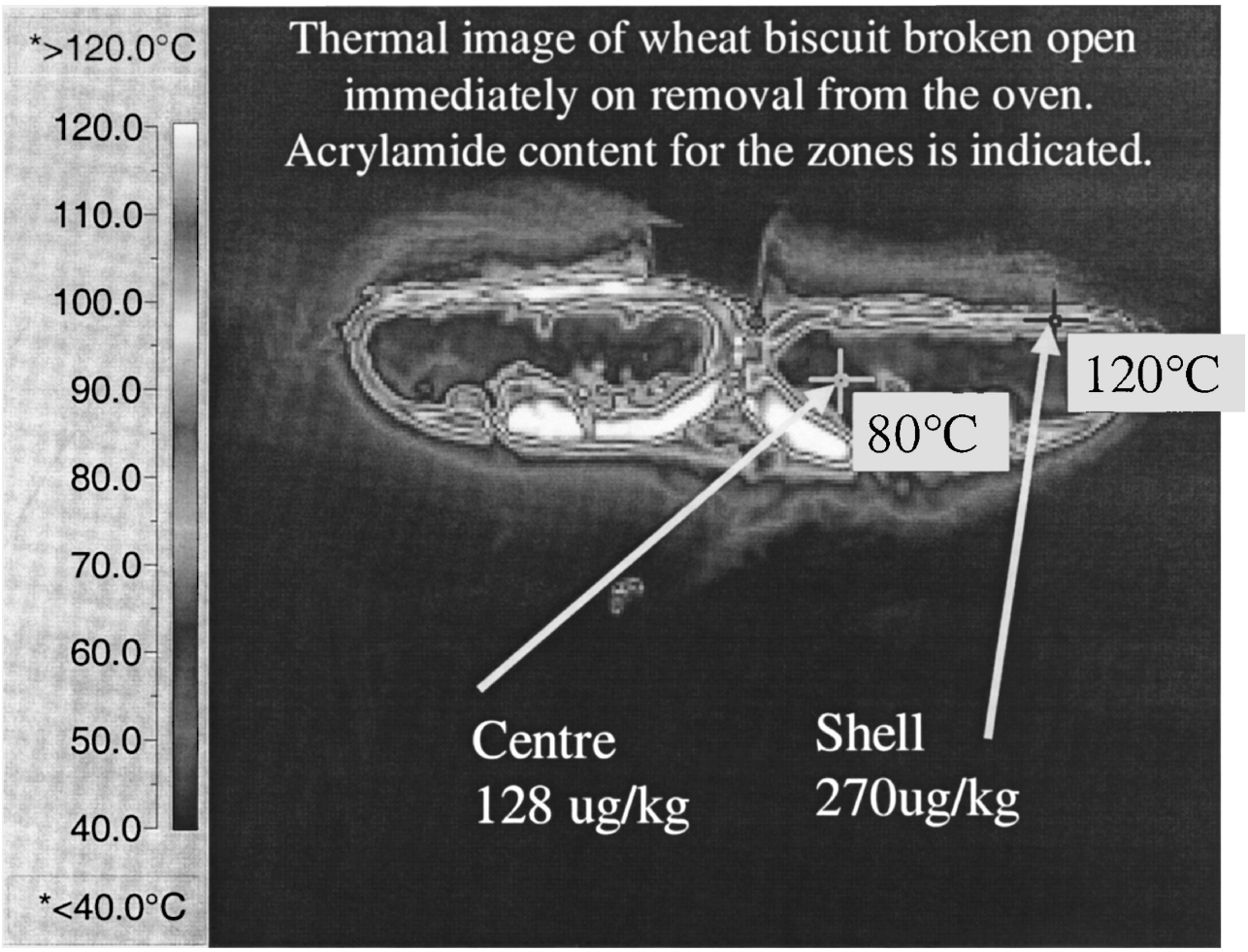


Figure 14 Thermal image of a cross section of wheat biscuit cereal at a point half way through an oven. The cooler centre zone never exceeds 80°C. The acrylamide content of the major temperature zones is indicated for a fully baked biscuit.

center. However, acrylamide is present in both zones of the biscuit. There is 270 μg/kg at the surface and 128 μg/kg in the cooler centre. When biscuits are toasted to the lowest degree compatible with edibility, the acrylamide concentration is increased by 15 to 45%. When biscuits are toasted to a near burnt state, the acrylamide concentration is decreased by 40 to 50%. Similar results have been shown for several other forms of cereal. They contrast with those for the potato, but are compatible with the suggestion made elsewhere that the acrylamide content results from a balance between formation and elimination, with the latter being more rapid at higher temperature.

In trials conducted so far, most (>90%) of the acrylamide forms when products are toasted. However, if the conditions of cooking the cereal are varied and the toasting is kept constant, then variations in the acrylamide content of the cereal may be shown (Table 8). The experiment was made under extreme conditions in respect of reducing sugars and, at present, is not a practical way to control acrylamide in edible food. It does, however, suggest that a precursor formed in the cooking stage may be made in variable amounts and converted to acrylamide at toasting. Thus, it would be a mistake to focus attention only on the toaster; the wet cooking stage may offer at least as much potential for the control of acrylamide content.

For cereal and other model systems, those spiked with asparagine generate more acrylamide than controls. In terms of the real process, knowledge of the "normal" range of free asparagine content for cereals is important. Table 9 summarizes the asparagine content for a range of European (mainly UK grown)

Table 8 Formation of acrylamide in wheat flakes when toasted under identical conditions

	Cooker cycle (minutes)		
Cooker pressure	Without reducing sugar	With reducing sugar added	Acrylamide (μg/kg)*
20 p.s.i/127°C	68	0	224
20 p.s.i/127°C	50	18	220
20 p.s.i/127°C	34	34	676
14 p.s.i/121°C	80	18	893

*Single values.

Table 9 Asparagine content of a selection of European wheat varieties

Wheat variety	AspN mg/kg	Origin*	Wheat variety	AspN mg/kg	Origin*
Abbot	329	UK	Hunter	151	UK
Abbot	334	UK	Isengrain	199	France
Abbot	344	UK	Kris	174	Germany
Abbot	664	UK	Malacca	176	UK
Abbot	344	UK	Mercia	168	UK
Admiral	206	UK	Mercia	210	UK
Apollo	214	UK	Napier	214	UK
Avalon	266	UK	Option	187	UK
Buster	295	UK	Orvantis	145	France
Caphorn	150	France	Piko	256	Germany
Chablis	102	UK	Rialto	178	UK
Charger	192	UK	Rialto	244	UK
Claire	202	UK	Rialto	251	UK
Claire	232	UK	Rialto	277	UK
Claire	163	UK	Rialto	286	UK
Consort	153	UK	Savannah	213	UK
Consort	273	UK	Shamrock	175	UK
Consort	302	UK	Shango	443	UK
Consort	326	UK	Slejpner	274	UK
Equinox	163	UK	Soissons	226	France
Folio	198	France	Tremie	179	France
Frelon	113	France	Vault	74	UK
Hereward	219	UK			

wheat varieties (measurements of asparagine were conducted by Jon Devries and John McKeehen of General Mills Inc.; Dr. Peter Jack of Monsanto UK kindly supplied the wheat samples). Between varieties there is a five-fold range: within the varieties, the limited data show at least a two-fold range. Notably, these samples represent a single crop year (2002), and many qualities of wheat vary significantly from one crop year to the next. There is no reason not to expect such variation for asparagine.

In summary, the food manufacture experiments show that increased toast temperature is not invariably associated with increased acrylamide. They raise the possibility that a precursor of acrylamide (e.g., *N*-glycoside) forms in the cooking stage for cereal. The asparagine content of wheat may be variable in respect to both variety and growing conditions. More work is needed to evaluate the practicality and value of growing crops with a minimal content of asparagine.

For the future, a clearer fundamental understanding of mechanisms and kinetics of acrylamide formation is essential, ideally over the range of temperatures and water concentrations involved in food processing. This knowledge would guide the design of process scale trials.

Biscuits

Industrial baking trials have been conducted on commercial products to investigate the effect of various parameters on acrylamide formation. Biscuits from the same production batch were sampled from the production line in order to evaluate average amounts of acrylamide. Each analysis was carried out on a homogenized subsample prepared from a single packet of biscuits.

Table 10 Variation of acrylamide levels in commercial biscuit products

Product	n	Range (μg/kg)	Average (μg/kg)	RSD %
Whole wheat biscuit	13	240 to 560	435	20
Plain biscuit	9	170 to 430	324	27

The results showed high intra-batch variations in acrylamide content (Table 10).

Ingredients play an important role in acrylamide formation, as different ingredients have various amounts of free asparagine and reducing sugars available for the formation reaction. Empirical reformulation trials have been carried out on commercial products to investigate which ingredients may influence acrylamide formation. The addition of whole wheat flour and bran to biscuit formulas tended to increase acrylamide in comparison with plain counterparts. Reducing the amount of the raising agent, ammonium bicarbonate, in formulas lowered acrylamide in plain flour matrices. The addition of lactic acid also lowered acrylamide content in plain flour matrices (from 195 μg/kg to 120 μg/kg; averages of two independent measures on independent trials).

The kinetics of acrylamide formation in biscuits have been studied. In agreement with other studies, there is no acrylamide present in uncooked dough but the acrylamide level rises rapidly with time. Temperature, cooking time, and final moisture condent are closely related in the baking process. Moisture content is an important factor for food preservation, but also for consumer acceptance.

Trials have been conducted on a model biscuit system to evaluate acrylamide formation in relation to the temperature and moisture content (Table 11 and Figure 15). All results are an average of 3 independent measurements and are shown in μg/kg product. At 10% moisture, no acrylamide was observed under the conditions employed; at 6% moisture, acrylamide was detected at the higher baking temperatures, but at moderate amounts. At a moisture content of 2%, acrylamide ranged from 165 to 363 μg/kg, depending on the baking temperature/time profile. The normal moisture content for commercial biscuits is $\sim$2%. The results demonstrated a relationship between color generation and acrylamide formation. Experiments are ongoing to determine the relative impact of baking temperature, baking time, final moisture content, and biscuit thickness on acrylamide formation.

Table 11 Acrylamide concentrations (μg/kg)* in model biscuits in relation to baking temperature and final moisture content

	Final moisture content		
Temperature/°C	10%	6%	2%
120	<20	<20	165
160	<20	28	229
200	<20	39	363

*Averages of n = 3 independent determinations.

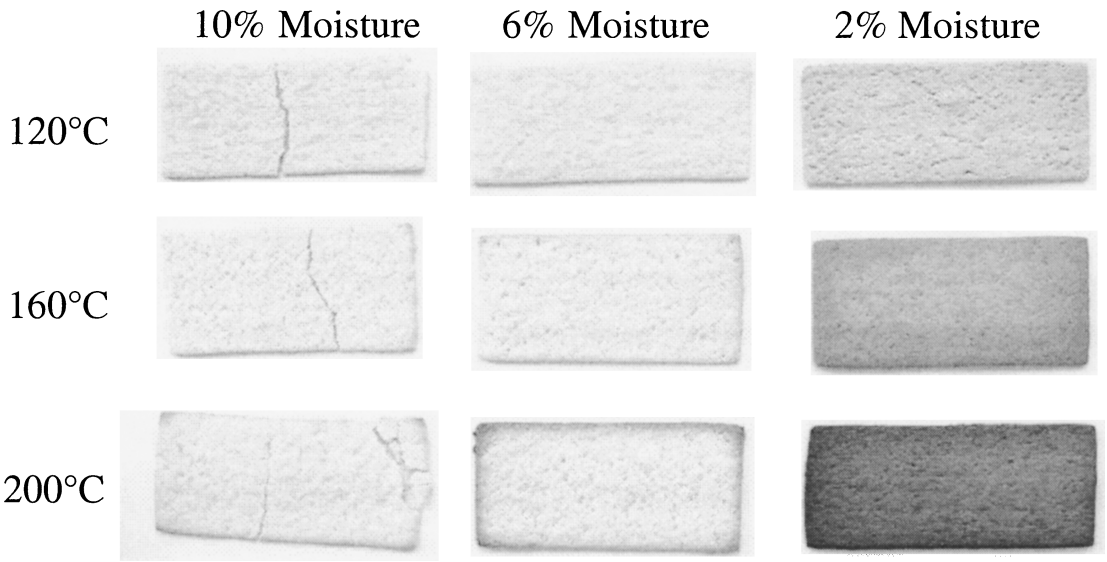


Figure 15 Comparison of model biscuits baked at various temperatures and with different moisture contents.

Coffee

Compared to the many other fried, roasted, and baked food products, roasted and ground coffee has been reported to contain relatively low concentrations (170–351 μg/kg) of acrylamide (Friedman, 2003). Furthermore, the dietary contribution of coffee to the total acrylamide intake varies (JIFSAN/NCFST Workshop, 2002; Konings et al., 2003; Svensson et al., 2003; Swiss Federal Office of Public Health, 19.12.2002). In addition, more uncertainty has been linked to the analytical methods established so far, due to the complexity of the matrix and difficulty to achieve reliable analytical data (Joint European Commission Workshop, 2003).

Recent studies using stable isotope labelled compounds have been conducted on the elimination of acrylamide in potatoes, showing that acrylamide declines fairly rapidly within the first 10–15 m at temperatures of approximately 120°C (Biedermann et al., 2002b). This effect is more pronounced in coffee that is roasted at temperatures at or above 220°C. A typical profile of formation of acrylamide at different roasting temperatures is illustrated in Figure 16. The amount of acrylamide measured increases exponentially at the onset of roasting, reaching an apparent maximum, and then decreasing rapidly as the rate of degradation exceeds the rate of formation. Experiments with deuterium labelled acrylamide spiked to green coffee prior to roasting confirm this behavior.

Therefore, light roasted coffees may contain relatively higher amounts of acrylamide than very dark roasted beans. The temperature *per se*, however, did not show a significant difference in the formation of acrylamide. Toward the commercial roasting (color) range, the acrylamide level was reduced by a factor of approximately 10, compared to the highest level recorded during the complete roasting cycle. Investigations are now ongoing to determine the impact of the coffee species/variety, the importance of free asparagine, as well as processing parameters on acrylamide formation.

OTHER MAILLARD-DERIVED COMPOUNDS OF POTENTIAL CONCERN: 3-BUTENAMIDE?

Very shortly after the reports of acrylamide in foods and first ideas on its formation, hypotheses on the presence of other vinyl compounds in cooked foods by an analogous route were established, such as 3-butenamide (Weisshaar and Gutsche, 2002). A salient feature in the Maillard reaction under low moisture conditions, shown recently (Yaylayan et al., 2003) and in this review, is that the route to the vinylogous products branches off at a very early stage of the Maillard reaction. This step encompasses an intramolecular cyclization of the Schiff base to afford an oxazolidine-5-one intermediate, which provides favorable conditions for decarboxylation at relatively low temperature (Manini et al., 2001) and subsequent β-elimination of the decarboxylated Amadori product.

Consequently, the composition of the food raw material (free amino acid pool and carbonyl source) will be an important determinant in predicting the corresponding vinyl product in cooked or thermally processed food (Stadler, 2003). For example, glutamine heated at moderate temperatures together with carbonyls or sugars could lead to the formation of 3-butenamide, and similarly glutamate to the formation of vinylacetic (3-butenoic) acid (Figure 17). The release of acrylic acid from sugar-aspartic acid binary mixtures has already been demonstrated (Stadler et al., 2003).

In theory, the thermal decomposition of sugar-glutamine condensates could similarly lead to the formation of 3-butenamide. Initial experiments were thus conducted to monitor 3-butenamide in sugar-glutamine pyrolysates using an LC-MS assay, choosing the characteristic transitions m/z 86 $\rightarrow$ 69, 86 $\rightarrow$ 44, 86 $\rightarrow$ 41 for 3-butenamide, corresponding to the fragments $[M+H\text{-}NH_3]^+$, $[CONH_2]^+$, and $[C_3H_5]^+$, respectively. However, in all model experiments with hexose-glutamine, a compound chromatographically separated from 3-butenamide

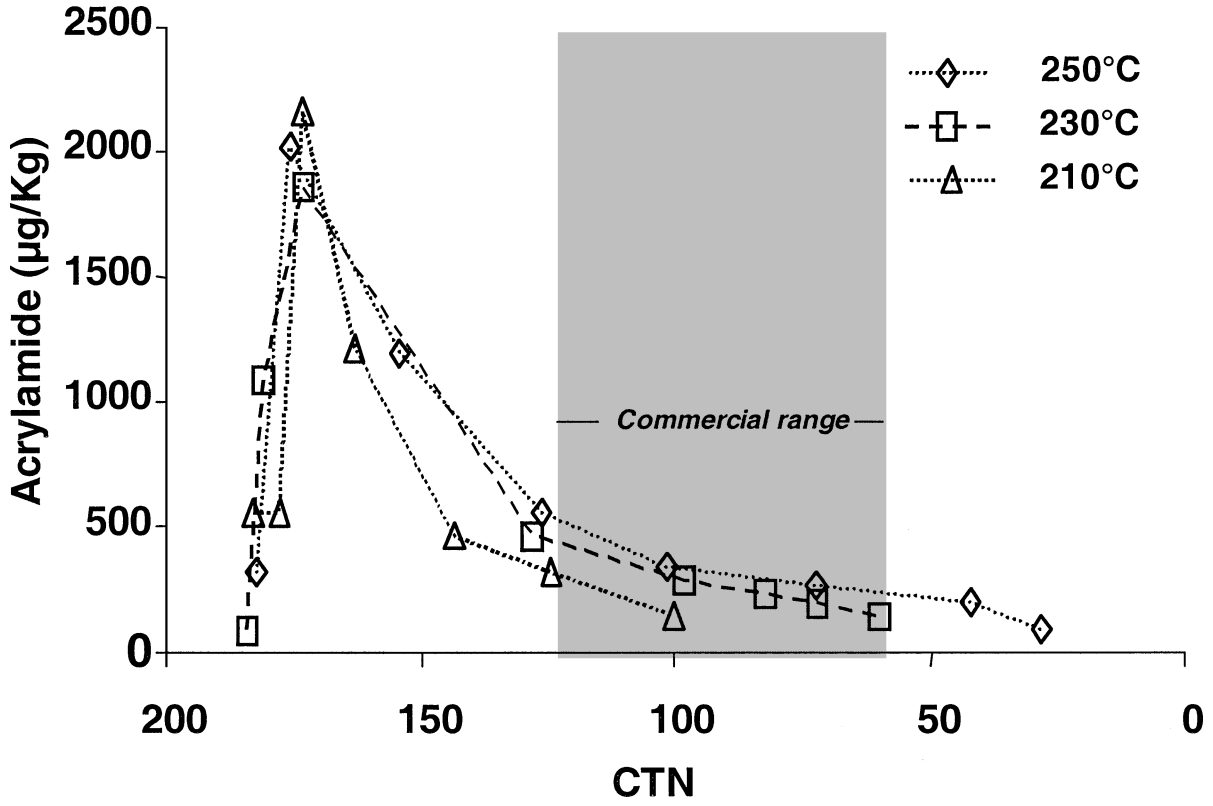
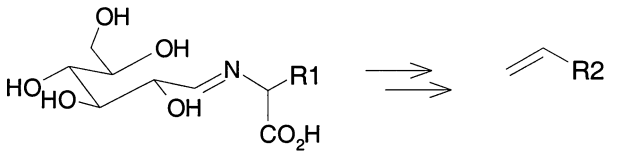


Figure 16 Formation of acrylamide in roast and ground coffee roasted at three different temperatures over time. CTN = color test number.

was observed. Moreover, the ion ratios of the reaction product were clearly different from those recorded for the standard 3-butenamide, indicating a molecule of related structure. Furthermore, 3-butenamide was only detected in spurious amounts (3.5–7.5 μmol/mol amino acid) at relatively low temperatures after heating sugar-glutamine mixtures in the temperature range of 120–140°C. The results were not reproducible.

Further characterization by high resolution mass spectrometry of the reaction products revealed that sugar-glutamine pyrolysates preferably release 2-pyrrolidinone. The LC-MS characteristics, including fragmentation pattern under MS/MS conditions, ion ratios, and retention time on the LC column, compared well with authentic 2-pyrrolidinone. Furthermore, the yield of 2-pyrrolidinone is comparable to that observed for acrylamide under the same reaction conditions (temperature, time, molar concentration of reactants). As shown in Table 12, thermolysis of fructose-glutamine or fructose-glutamate mixtures efficiently release 2-pyrrolidinone (>4 mmol/mol amino acid, not corrected for recovery) at 240°C, after a heating period of 5 m. This is in contrast to recent work done by Biedermann et al. (2003), which could not detect appreciable yields of 2-pyrrolidinone in their models during the co-pyrolysis of reducing sugars and glutamine or glutamic acid. In our experiments, glutamine and glutamate both follow similar temperature kinetics, supporting a common mechanism to the formation of 2-pyrrolidinone.



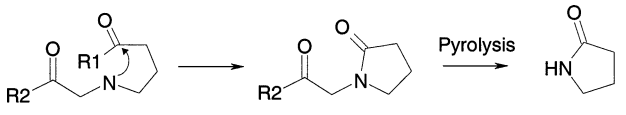
Asn: R1 = CH_2-$CONH_2$; R2 = $CONH_2$
Asp: R1 = CH_2COOH; R2 = COOH
Gln: R1 = CH_2-CH_2-$CONH_2$; R2 = CH_2-$CONH_2$
Glu: R1 = CH_2-CH_2-COOH; R2 = CH_2-COOH

Figure 17 Possible formation of vinylogous compounds from the corresponding amino acids/sugars in the Maillard reaction.

Table 12 Formation of 2-pyrrolidinone (mmol/mol ± SD)* from fructose-Gln and fructose-Glu co-pyrolysates (each 0.2 mmol of reactants, 5 m heating period, closed systems

Temperature (°C)	Fructose-Gln	Fructose-Glu
160	0.27 ± 0.028	0.03 ± 0.003
170	0.46 ± 0.044	0.16 ± 0.06
180	0.81 ± 0.13	0.15 ± 0.02
190	0.91 ± 0.36	0.41 ± 0.05
200	1.39 ± 0.12	1.32 ± 0.12
210	1.48 ± 0.16	2.38 ± 0.23
220	1.82 ± 0.22	3.15 ± 0.45
230	3.59 ± 0.61	3.08 ± 0.16
240	4.25 ± 0.19	4.07 ± 0.26

*all entries are averages of n = 3 independent determinations.



Gln: R1 = NH_2; R2 = $C_4H_9O_4$
Glu: R1 = OH; R2 = $C_4H_9O_4$

Figure 18 Proposed formation of 2-pyrrolidinone in the Maillard reaction from glutamine-sugar or glutamate-sugar.

Accordingly, thermal degradation of carbonyl-glutamine and carbonyl-glutamate mixtures could lead to the formation of 3-butenamide and 3-butenoic acid, respectively. These reactions are apparently not favored, and both amino acids release significant amounts of 2-pyrrolidinone as a major degradation product. Thermally catalyzed intramolecular cyclization, probably of the decarboxylated glycosylamine (Figure 18) followed by heterolytic cleavage, could release 2-pyrrolidinone, the sugar facilitating ring closure and the scission of the C—N bond. Comparable temperature kinetics of formation of 2-pyrrolidinone from both glutamine and glutamate co-pyrolyzed with hexose sugars support a common mechanism.

The previously suspected 3-butenamide is only present in spurious amounts in the model systems and is probably of much less importance in cooked foods (Biedermann et al., 2003; CIAA/UK FSA/Dutch VWA Workshop, 2003; Stadler et al., 2003). Furthermore, 3-butenamide is relatively stable under moderate temperatures and does not cyclize to 2-pyrrolidinone. Therefore, the formation of corresponding vinylogous compounds in foods is not clear cut and must be studied on a case-by-case basis, taking into account to the availability of free amino acids and sugars in the raw agricultural commodity and conditions of thermal processing. Based on these findings, the Maillard reaction may be a major contributor to 2-pyrrolidinone in cooked and roasted foods and would principally be detectable in all foods with free glutamine/glutamate, a carbonyl source, and those that are heated at moderate temperatures and under low moisture conditions.

FUTURE RESEARCH EFFORTS AND CHALLENGES AHEAD

The WHO/FAO convened jointly a consultation on the health implications of acrylamide in food (FAO/WHO Consultation, 2002). The consultation concluded that the presence of acrylamide in food is a major concern for humans, based on the ability to induce cancer and heritable mutations in laboratory animals. Both the WHO and FAO acknowledged that available data are too limited to allow more informed estimates of risk to humans. The consultation strongly recommended research on the following: (a) the methods of analysis (interlaboratory validation, etc.); (b) the mechanism(s) of formation and fate of acrylamide in food (relation between acrylamide and processing/cooking conditions, etc.); (c) the modes of absorption (bioavailability), metabolism, and excretion of acrylamide in humans by the oral route; (d) the cancer epidemiology in populations of high exposure. The consultation concluded that the creation of an international network (i.e., through a website) on acrylamide in food, intended as a global resource and inventory of ongoing research, would be the key to a proactive approach in acrylamide research. This would identify gaps and avoid overlaps in the planned and ongoing research and information, focusing the available resources and sharing data and information of all stakeholders in a transparent and open manner. In fact, two such sites have recently been established, the WHO/FAO Acrylamide Information Network, operated by JIFSAN (JIFSAN Infonet), and another listing and categorizing all research on acrylamide planned/ongoing in the European Union (Acrylamide in Food—Database of Activities in the EU). Collaboration on the operation of the two sites (JIFSAN and EFSA) is occurring to ensure that as many projects as possible are listed in the Acrylamide Infonet.

The European Commission's JRC is coordinating work on analytical methods and is collecting data on the concentrations found in different foods. The activities of the JRC will include interlaboratory comparison studies conducted by IRMM in order to discover sources of errors within the sample preparation steps and measurement, respectively. To aid in improving method performance, the IRMM of the JRC will also carry out feasibility studies on the production of reference acrylamide materials. The JRC has established a task force consisting of experts in the field to further assess analytical methods.

For long term research needs, the European Commission has included the topic in its 6th Framework Programme for Research and Technological Development. Similarly, the United States Food and Drug Administration (FDA) has prepared an action plan for acrylamide in food, outlining the research goals and activities over the next years (FDA Draft Action Plan for Acrylamide in Food). So far, over 100 research projects have been registered on these websites, and several international meetings have already been held with experts in the field, from the private sector, government, and academia, to "informally" exchange progress in the science (JIFSAN/NCFST Workshop, 2002; Joint European Commission Workshop, 2003).

Significant scientific progress has been achieved over the past twelve months in understanding the chemistry of acrylamide (Friedman, 2003). First, analytical tools to measure acrylamide in many different types of food have been developed and shared amongst the analysts (Joint European Commission Workshop, 2003). Some problems that are highlighted in this review remain, but they are being addressed in a cooperative manner. Second, the main mechanisms for the formation of acrylamide in foodstuffs have been identified, which has contributed to the understanding of acrylamide amounts found in foods and the impact of food processing on acrylamide. This offers ways of bringing about a reduction in exposure, e.g., through agronomical measures, technological changes, or a combination of both. In fact, in some areas, the concentration of acrylamide could be reduced, as recently highlighted in a workshop held by the European Commission (EC Acrylamide Workshop,

20–21 October 2003). Representatives of the EU Member States, different sectors of the food and catering industries, and consumers attended this meeting. A note generated from this event highlights ways to lower levels of acrylamide formed in food and indicates a number of provisional recommendations for producers, processors, retailers, caterers, and consumers. Therefore, certain approaches can already be used to help to reduce the formation of acrylamide in food, during commercial production, processing and preparation, as well as during preparation in the home.

Despite the recommendations made by WHO/FAO and endorsed by European Commission Scientific Committee on Food (SCF) in their opinion of 3rd July 2002, the acrylamide issue is being approached differently by governments. For example, Germany has introduced a signal value based on the minimization principal (Künast, 2002). This involves identifying the categories of food products among the 10% with the highest concentration of acrylamide. Once these categories are chosen, the "signal value" per category will be fixed (i.e., 90th percentile of each category). The aim is to reduce dynamically and progressively the acrylamide contents in industrially processed foods. This may, however, not be as simplistic as outlined. Since for many foods, "quick fixes" will not be possible, necessitating intensive research on the agronomical and processing side. Depending on the food product, its raw materials, ingredients, and complexity in the processing stages, practical and economically feasible solutions may be difficult or even impossible to devise. Nevertheless, the food industry acknowledges its share of the responsibility in understanding how acrylamide is formed and in identifying what measures (agronomical, technological) can be taken to reduce acrylamide without compromising the product quality and consumer acceptability or generating further "unknown" contaminants of potential health concern.

The acrylamide issue is not restricted to industrially processed food, and the contribution of intake via domestic cooking, out-of-home eating and catering, may be significant. A random survey by the German BfR related to the dietary habits of more than 1,000 average 16-year-olds in Berlin revealed that more than 20% of the average daily intake of acrylamide came from fried potatoes (7%) and toast (15%). The Institute has also distributed a flyer "Acrylamide—how to protect yourself and your family," with information on how to reduce the formation of acrylamide in the home. The knowledge gained by industry can be shared and in some instances may be applied to devise concrete measures to "lower" exposures to acrylamide from home cooking practices. Any proposed measures must, however, take into account the overall effects that changes in cooking regimes may have. For example, possible increases in fat content in French fries when heated at relatively lower temperatures, or loss of vitamins upon leaching out precursors.

During the past year researchers from industry, national authorities, the EU Commission and academia have gained increasing insight in understanding the presence, formation and potential risk to public health posed by the unexpected discovery of acrylamide in some foodstuffs. In spite of this endeavor, significant gaps remain in this knowledge base. On 28 March 2003, a workshop (CIAA/UK FSA/Dutch VWA Workshop, 2003) was held to discuss the current state of the science and knowledge, to identify the gaps and to recommend actions. This event highlighted the urgent need to coordinate research efforts internationally and establish a mechanism for information exchange. The formation of acrylamide in a wide range of both commercial production and domestic preparation processes was reviewed for various food groups. This demonstrated that, whereas some information is available on the reducing sugars and free asparagine content of the raw agricultural commodities (particularly potatoes and cereals) and how these constituents and the processing conditions influence the formation of acrylamide, much less is known about the effects of final preparation in food service and domestic situations. Laboratory research is currently focused on aspects of industrial processing and much less research is being conducted on aspects relating to the domestic and food service sectors. The development of an information exchange mechanism was deemed essential to facilitate rapid sharing of knowledge and experience between all researchers investigating acrylamide in food, thereby preventing unnecessary duplication of effort and allowing the rapid identification of gaps and overlooked topics. CIAA has already established such a group. This paper is the result of industry's pooling of knowledge and serves to demonstrate its commitment to playing its part in satisfactorily resolving the acrylamide issue.

Acrylamide is scheduled for evaluation by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at its 65th meeting in February 2005. A discussion paper has been prepared (ftp://ftp.fao.org/codex/ccfac36/fa36_34e.pdf) and the outcome of this risk assessment will provide a basis for further recommendations or potential legislative decisions. Finally, as recommended by WHO/FAO, SCF, and the U.S. FDA, people should not change their dietary habits and continue to eat a balanced diet rich in fruit and vegetables and moderate their consumption of fried and fatty foods. Many national authorities across the world are assessing the dietary exposure of consumers to acrylamide (Konings et al., 2003; Svensson et al., 2003), and scientific projects are being funded to gather new information about the toxicology of acrylamide. These are expected to provide new scientific knowledge that will help to clarify whether or not there is a risk to human health from the consumption of foods containing low amounts of acrylamide.

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