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1. Ludwig, A., May, B., Debus, L. & Jenneckens, I. *Genetics* **156,** 1933–1947 (2000).
2. King, T. L., Lubinski, B. A. & Spidle, A. P. *Conserv. Genet.* **2,** 103–119 (2001).
3. Brown, J. T., Beckenbach, A. T. & Smith, M. J. *Mol. Biol. Evol.* **10,** 326–341 (1993).
4. Magnin, E. *Le Naturaliste Canadien* **91,** 5–20 (1964).
5. Artyukhin, E. & Vecsei, P. *J. Appl. Ichthyol.* **15,** 35–37 (1999).
6. Borodin, N. *Trans. Am. Fish. Soc.* **55,** 184–190 (1925).
7. Quantz, H. *Mitt. Dt. Seefischerei-Vereins* **19,** 176–204 (1903).

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Food chemistry

Acrylamide is formed in the Maillard reaction

Reports of the presence of acrylamide in a range of fried and oven-cooked foods[1,2] have caused worldwide concern because this compound has been classified as probably carcinogenic in humans[3]. Here we show how acrylamide can be generated from food components during heat treatment as a result of the Maillard reaction between amino acids and reducing sugars. We find that asparagine, a major amino acid in potatoes and cereals, is a crucial participant in the production of acrylamide by this pathway.

Products of the Maillard reaction are responsible for much of the flavour and colour generated during baking and roasting. An important associated reaction is the Strecker degradation of amino acids by these intermediates (Fig. 1), in which the amino acid is decarboxylated and deaminated to form an aldehyde.

We investigated whether this reaction could provide a possible route to acrylamide. The amino acid asparagine should be a particularly suitable reactant as it already has an amide group attached to a chain of two carbon atoms. We therefore performed a series of Maillard reactions between glucose and asparagine, as well as with other amino acids that do not have the correct carbon backbone for acrylamide (Fig. 1).

Significant quantities of acrylamide (221 mg per mol of amino acid) were found when an equimolar mixture of asparagine and glucose was reacted at 185 °C in phosphate buffer in a sealed glass tube. The temperature dependence of acrylamide formation from asparagine indicates that this is favoured above 100 °C and that very high temperatures are not necessary (Fig. 2). In similar reactions with glucose and glycine, cysteine or methionine at 185 °C, no acrylamide was detected (detection limit, 0.5 mg mol^{-1}). Glutamine and aspartic acid gave only trace quantities of acrylamide (0.5–1 mg mol^{-1}).

When a dry mixture of asparagine and glucose was reacted at 185 °C (that is, without buffer solution), only 25 mg mol^{-1} acrylamide was formed. Although the dry reaction is a realistic system with which to simulate the later stages of baking and toasting of food, it is less efficient because the reactants are incompletely mixed in the absence of a solvent. Trace quantities of acrylamide were produced under these conditions from glutamine and aspartic acid, but not from any of the other amino acids apart from methionine, which yielded 5 mg mol^{-1}.

To test for the involvement of Strecker degradation in the the production of acrylamide, we used 2,3-butanedione instead of glucose in these reactions (butanedione is one of several dicarbonyl compounds formed in the Maillard reaction). Acrylamide was produced when asparagine was allowed to react with butanedione both in a dry system (40 mg mol^{-1}) and in buffer (63 mg mol^{-1}). Heating asparagine on its own at 185 °C did not produce acrylamide, confirming the requirement for the dicarbonyl reactant and Strecker degradation.

Again, there was no significant production of acrylamide in either system from butanedione and the other amino acids, with the exception of methionine (6 mg mol^{-1} in the dry system). The Strecker aldehyde formed from methionine is methional, but acrolein can also be formed, together with ammonia: subsequent oxidation of acrolein to acrylic acid followed by amidation could then generate acrylamide (Fig. 1). However, this reaction might be limited by its requirement for ammonia, which reacts readily with carbonyls and other Maillard intermediates.

The almost exclusive formation of acrylamide from asparagine could explain the occurrence of acrylamide in cooked plant-based foods, such as cereals and potato, which are rich in this particular amino acid[4]. In potato used for the manufacture of potato crisps, the dominant free amino acid is asparagine (940 mg kg^{-1}, representing 40% of the total amino-acid content[5]); in wheat flour it is present at 167 mg kg^{-1}, corresponding to 14% of the total free amino acids (our unpublished results), and a high-protein rye variety contains 173 mg kg^{-1} (18% of the total free amino acids)[6].

Our findings indicate that Maillard reactions involving asparagine can produce acrylamide and might explain the increased concentrations of acrylamide in certain plant-derived foods after cooking.

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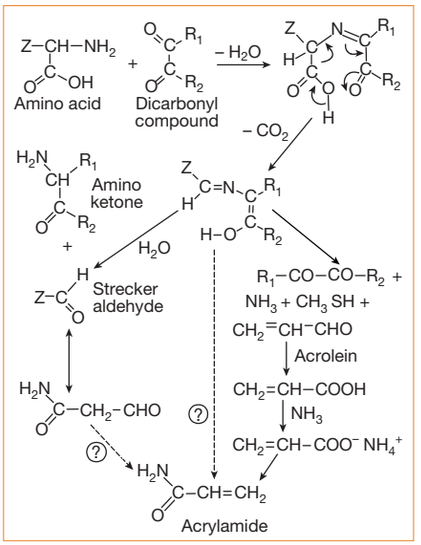

Figure 1 Proposed pathways for the formation of acrylamide after Strecker degradation of the amino acids asparagine and methionine in the presence of dicarbonyl products from the Maillard reaction. In asparagine, the side chain Z is $–CH_2CONH_2$; in methionine, it is $–CH_2CH_2SCH_3$.

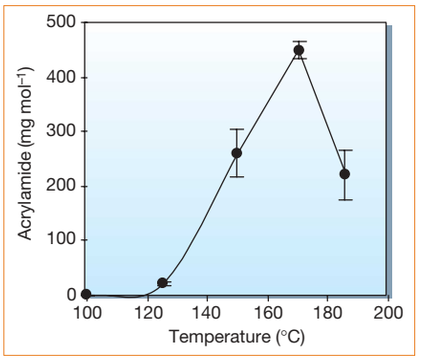

Figure 2 Temperature-dependent formation of acrylamide (mg per mol of amino acid) from asparagine (0.1 mmol) and glucose (0.1 mmol) in 0.5 M phosphate buffer (100 ml, pH 5.5) heated in a sealed glass tube for 20 min. Error bars represent standard deviations (n 3). Acrylamide produced in the reaction was extracted with ethyl acetate and analysed by gas chromatography with mass spectrometry after derivatization to 2,3-dibromopropanamide[7], using 2-methylacrylamide as the internal standard. Selected ion monitoring was used to detect the analytes, with m/z 150 and 152 for acrylamide and m/z 120 and 122 for methylacrylamide. The presence of acrylamide in selected samples was confirmed in full mass spectra.

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1. Rosen, J. & Hellenas, K.-E. *Analyst* **127,** 880–882 (2002).
2. Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S. & Törnqvist, M. *J. Agric. Food Chem.* **50,** 4998–5006 (2002).
3. IARC *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* **60,** 389 (1994).
4. Belitz, H.-D. & Grosch, W. *Food Chemistry* (Springer, New York, 1999).
5. Martin, F. L. & Ames, J. M. *J. Agric. Food Chem.* **49,** 3885–3892 (2001).
6. Dembinski, E. & Bany, S. *J. Plant Physiol.* **138,** 494–496 (1991).
7. Castle, L. *J. Agric. Food Chem.* **41,** 1261–1263 (1993).

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Food chemistry

Acrylamide from Maillard reaction products

The discovery of the adventitious formation of the potential cancer-causing agent acrylamide in a variety of foods during cooking[1,2] has raised much concern[3,4], but the chemical mechanism(s) governing its production are unclear. Here we show that acrylamide can be released by the thermal treatment of certain amino acids (asparagine, for example), particularly in combination with reducing sugars, and of early Maillard reaction products (*N*-glycosides)[5]. Our findings indicate that the Maillard-driven generation of flavour and colour in thermally processed foods can — under particular conditions — be linked to the formation of acrylamide.

We heated 20 amino acids individually at 180 °C for 30 min and found that acrylamide is formed under these conditions from methionine and from asparagine (3.651.4 and 0.5650.05 μmol acrylamide per mol amino acid, respectively; all results are averages of *n*46 independent determinations unless stated otherwise).

When pyrolysed at 180 °C with an equimolar amount of glucose, asparagine in particular generates significant amounts of acrylamide, reaching an average of 368 m mol mol^{11} after an incubation time (t_i) of 30 min. If asparagine monohydrate was used in the incubation or water was added to the reaction (0.05 ml) before thermolysis, the release of acrylamide was enhanced nearly threefold (9605210 m mol mol^{11}), or over 1,700 times the amount formed from asparagine alone under the same conditions.

Reaction of methionine and glutamine with equimolar amounts of glucose at 180 °C also increased the formation of acrylamide, which occurred rapidly in each case (t_i45 min; Fig. 1a). Cysteine was found to liberate acrylamide after condensation with glucose (2.050.8 m mol mol^{11} at t_i430 min and 180 °C).

Investigating the role of different carbohydrates in the formation of acrylamide, we found that pyrolysing any of these amino acids (Asn, Gln, Met, Cys) with an equimolar amount of D-fructose, D-galactose, lactose or sucrose all led to a significant release of acrylamide, with comparable yields from each sugar. No acrylamide was detected when any of these carbohydrates was heated alone.

To test whether early Maillard products such as *N*-glycosides could be acrylamide precursors in thermal decomposition reactions, we measured the yields of acrylamide after pyrolysis (t_i420 min, 180 °C) of 0.2 mmol of four different *N*-glycosides (Fig. 1b). Yields were significant (in m mol per mol *N*-glycoside: compound **1**, 1,3055323; **2**, 1,4195278; **3**, 1452.7; and **4**, 8.151.5) and comparable to those released from the amino-acid and reducing-sugar precursors under the same conditions. Furthermore, compound **1** was confirmed as an intermediate in the asparagine/glucose reaction by high-resolution mass-spectrometric analysis of a methanol extract of the pyrolysate.

On the basis of structural considerations, asparagine or the *N*-glycosides **1** and **2** could be direct precursors of acrylamide under pyrolytic conditions. Condensation of asparagine with $^{13}C_6$-labelled glucose confirmed that the amino acid is the carbon source of acrylamide. Upon pyrolysis, formation of the corresponding *N*-glycoside probably facilitates the decarboxylation step and heterolytic cleavage of the nitrogen–carbon bond to liberate acrylamide ($CH_25CHCONH_2$). Although decarboxylation is favoured at higher temperatures, the *N*-glycosidic bond seems to facilitate the deamination step.

Further evidence to support this pathway to acrylamide production is provided by the 98.6% incorporation of nitrogen-15 label into acrylamide after the pyrolysis of ^{15}N-amide-labelled asparagine with glucose; there was no incorporation into acrylamide when ^{15}N-a-amino-labelled asparagine was used in the same reaction. Results from similar isotope-labelling experiments (not shown) to determine the route of acrylamide formation from different *N*-glycosides produced by glucose pyrolysis with glutamine or methionine are less clear-cut, which suggests that other pathways (such as that for homolytic cleavage) might also lead to acrylamide.

The *N*-glycosidic bond is labile in the presence of water[6] or under acidic and neutral pH conditions[7], hydrolysing rapidly to the reducing sugar and amino acid. At higher pH, however, *N*-glycosides can be isolated as bimolecular complexes in the presence of polyvalent alkaline or transition-metal ions[8]. In food-processing systems that incorporate conditions of high temperature and water loss, *N*-glycoside formation could be favoured; when this condensation occurs between reducing sugars and certain amino acids, a direct pathway is opened up to potential progenitors of acrylamide.

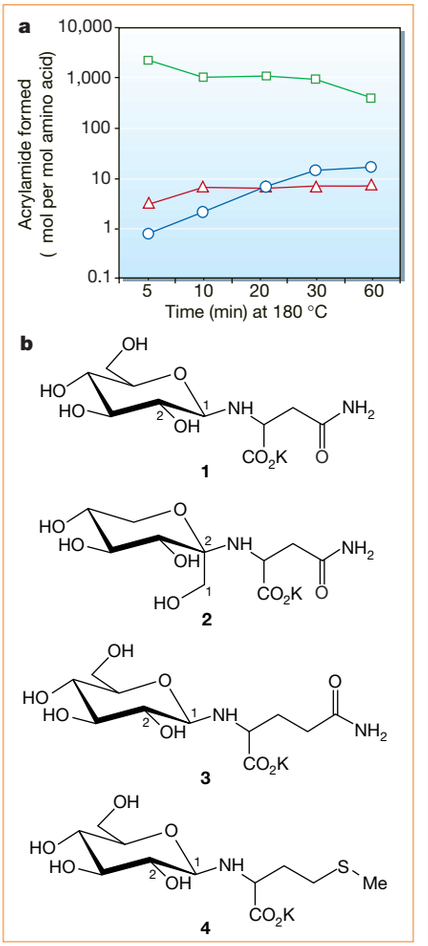

Figure 1 Production of acrylamide from *N*-glycosides. **a,** Logarithmic-scale plot of the formation of acrylamide over time in pyrolysates of glucose with glutamine (triangles), asparagine (squares) or methionine (circles). Each data point represents the average of *n*43 independent determinations; the coefficient of variation was less than 25%. For acrylamide analysis (by liquid chromatography coupled to electrospray ionization tandem mass spectrometry), pyrolysates were supplemented with $^{13}C_3$-acrylamide (50 ng), then suspended in hot water (more than 90 7C), sonicated and filtered before being applied to a solid-phase extraction cartridge (OASIS HLB, 0.2 g). Acrylamide eluted with 20% methanol was separated on a Shodex RSpak DE-613 polymer column with isocratic solvent flow. Detection by mass spectrometry was in the multiple-reaction monitoring mode with the characteristic fragmentation transitions for acrylamide (*m/z* 72→55, 72→27, 72→54) and confirmed by ion ratios (55/54 and 55/27). Further details are available from the authors. **b,** Chemical structures of the potassium salts of *N*-(D-glucos-1-yl)-L-asparagine (**1**), *N*-(D-fructos-2-yl)-L-asparagine (**2**), *N*-(D-glucos-1-yl)-L-glutamine (**3**) and *N*-(D-glucos-1-yl)-L-methionine (**4**).

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1. Swedish National Food Agency website http://www.slv.se
2. Rosén, J. & Hellenäs, K.-E. *The Analyst* **127,** 880–882 (2002).
3. WHO *FAO/WHO Consultation on the Health Implications of Acrylamide in Food* (Geneva, 25–27 June 2002)