

Tests for the Depolymerization of Polyacrylamides as a Potential Source of Acrylamide in Heated Foods

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This study was conducted to test the possibility that polyacrylamides used in agriculture may contribute to acrylamide formation in heated foods by thermal depolymerization. Two samples of polyacrylamide with low molecular weights of 1.5 and 10 kDa were used to test for in-chain and end-chain depolymerization. They were added as aqueous solutions to filter paper for heating in a drying/dry environment and added to a cooking oil for heating in a fatty environment. The heating conditions were 175 °C for 15 and 30 min, respectively. Both regimens were tested in the absence and presence of the redox-active metal ions Fe(III) and Cu(II), and all tests were conducted without the exclusion of atmospheric oxygen. There was no evidence of any significant depolymerization of polyacrylamide to free acrylamide monomer, <0.04%. In fact, residual levels of acrylamide present already in the low molecular weight polymers were seen to fall by 50–80% on heating. Consequently, it is concluded that even if polyacrylamides were to contaminate agricultural crops and foods derived therefrom (which itself is an unproven suggestion), there is no evidence that the polymers would depolymerize on heating of the food to form acrylamide in any significant amount.

KEYWORDS: Acrylamide; polyacrylamide; thermal depolymerization; degradation; heated foods

INTRODUCTION

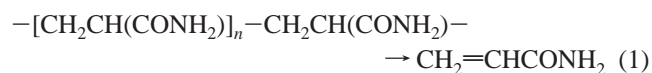
Following the unexpected discovery that acrylamide (CH₂=CHCONH₂) is formed in some heated foods (1–3), efforts worldwide focused quickly on identifying the precursor(s) and mechanism(s) of formation of this toxic substance. With impressive speed and insight, a major route to formation was postulated and demonstrated to be the reaction of the amino acid asparagine with reducing sugars (4, 5). To date, this is the only important route of formation demonstrated, although many others have been suggested (6, 7), and it remains to be seen if other precursors and reactions make any significant contribution to acrylamide formation in heated foods.

One possible route for the formation of free acrylamide was proposed to be from the degradation of polyacrylamides that are used in agriculture (8). Polyacrylamides are used in large quantities in agriculture, for example, as an adjunct in pesticide formulations to reduce spray drift and give a more uniform spray pattern and as a wetting agent to increase the contact time with the plant (9). It had been reported (10, 11) that polyacrylamide in the presence of glyphosate degraded to free acrylamide monomer by a free radical mediated process initiated by light. The validity of these findings was questioned (12), and subsequent work in another laboratory failed to reproduce the findings and reported that polyacrylamide did not degrade to acrylamide monomer in the presence of sunlight or glyphosate or with any combination of the two (9).

In thermogravimetric analysis (TGA), when dried samples of polyacrylamide were heated in an inert atmosphere in the temperature range of 30–600 °C, the first stages of decomposition were not observed until 220–340 °C (13). This is well above the temperature of ~130 °C for the onset of acrylamide formation in heated foods. In the TGA tests, the polymer chains remained intact and reaction at the pendant amide groups led to the evolution of water, ammonia, and carbon dioxide. At higher temperatures, 340–440 °C, decomposition of the main chain occurred but depolymerization to form acrylamide was not identified as a significant reaction pathway. Similarly, flash pyrolysis of polyacrylamide at 500 °C identified acetonitrile, acrylonitrile, propionitrile, methacrylonitrile, and isobutyronitrile as the five major pyrolysis products, but not acrylamide (14).

Although these reports on the absence of evidence of depolymerization to form acrylamide monomer were reassuring, it has to be recognized that the reaction conditions differed considerably from those that pertain during the cooking of foods. Consequently, the suggestion that acrylamide is released from polyacrylamide in the environment and that cooking vegetables that had been exposed to the polyacrylamide in herbicides or used during soil preparation would result in the release of more acrylamide (8) could not be discounted.

In principle, any polymerization reaction can be driven backward (eq 1 for the depolymerization of polyacrylamide to form free acrylamide monomer). In practice, for addition



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polymers, re-forming the monomer is an energetically unfavorable process. Depolymerization can proceed significantly only if the process is made favorable by the entropy term in the Gibbs free energy of reaction, that is, at high temperatures. The only example of thermal depolymerization of a common plastic is polystyrene, which depolymerizes during thermal processing operations such as injection molding and thermoforming, such that styrene monomer levels are typically in the range of 200–400 mg/kg in the plastic and are difficult to reduce further. Additional heating can cause further depolymerization, so, for example, cooking trays made of unsaturated polyesters cross-linked with styrene, were found to depolymerize to form additional styrene when used to heat foods in an oven at >175 °C (15).

Our interest in the use of polyacrylamides in agriculture stems from our early work, which found that when polymers are used in hydroponics or as a water conservation agent, there was no detectable accumulation of acrylamide monomer in tomatoes or mushrooms, respectively (16, 17). We report here work conducted to investigate the thermal stability of polyacrylamides under the heating conditions that have been found to cause acrylamide formation in cooked foods.

EXPERIMENTAL PROCEDURES

Chemicals. Polyacrylamide, average molecular weight 1500 (1.5 kDa), 50% solution (PA1), and average molecular weight 10000 (10 kDa), 50% solution (PA2), were from Aldrich (Gillingham, U.K.). Small filter papers (4.5 cm diameter) were Whatman no. 1. Sunflower oil was purchased from a local supermarket. Iron(III) nitrate, nonahydrate (98+%), and copper(II) nitrate, hemipentahydrate (98%), were from Aldrich.

Heating Tests on Filter Paper (Dry Matrix). A filter paper disk was placed in a glass Petri dish. Polyacrylamide solution (10 μ L, 50%) was placed onto the paper surface and allowed to soak in. A lid was placed over the dish, which was then put in a preheated oven for 15 min at 175 °C. The sample was then removed from the oven and allowed to cool, and the paper disk was removed to a conical flask using tweezers. Ten milliliters of distilled water was used to rinse the internal surfaces of the Petri dish and the lid. The washings were added to the conical flask, and the filter paper was extracted by standing at room temperature for \sim 1 h with occasional swirling. A portion (5 mL) of the water extract was taken into a screw-cap vial and submitted to LC-MS analysis.

For tests in the presence of Fe(III) or Cu(II) ions, 100 μ L of the metal nitrate solution (1 g/L in distilled water) was added onto the filter paper and allowed to soak in. The polyacrylamide was then added onto the same region of the filter paper, which was then heated as described above.

Heating Tests in Sunflower Oil (Fatty Matrix). Sunflower oil (5.0 g) was placed into a screw-capped vial, and polyacrylamide solution (10 μ L, 50%) was added into the oil. The vial was capped to make it gastight, using a PTFE-faced septum, and shaken by hand to mix the contents. The sample was placed into a preheated oven for 30 min at 175 °C. The sample was then removed and allowed to cool, and then hexane (5 mL) and water (10 mL) were added followed by shaking to extract. The mixture was centrifuged to separate the layers, and a portion of the aqueous extract (5 mL) was taken into a screw-cap vial and submitted to LC-MS analysis.

Stability Tests in the Presence of Added Metal Ions. For tests in the presence of Fe(III) or Cu(II) ions, 100 μ L of the metal nitrate solution (1 g/L in distilled water) was added to the oil. The polyacrylamide was then added to the oil, which was then heated, etc., as described above.

Analysis by LC-MS/MS for Acrylamide. To the 5 mL portion of the aqueous extract was added 50 μ L of a 10 μ g/mL solution of *d*₃-acrylamide in water, as internal standard. The LC-MS/MS analysis for acrylamide was performed as described (18).

RESULTS AND DISCUSSION

Experimental Design. These tests were conducted to determine if typical polyacrylamides depolymerize on heating at temperatures experienced during frying or baking of foods. The tests were designed such that 5 mg of polyacrylamide was submitted to each heating regimen. Each test was conducted in duplicate. The sensitivity of the extraction and LC-MS procedure used was such that any decomposition to form $>0.04\%$ of free acrylamide would be detected and measured.

Polyacrylamides Chosen for Study. Polyacrylamides used in agriculture have a very high molecular weight, up to millions of daltons (MDa). We chose to use much lower MW polyacrylamides, PA1 at 1.5 kDa and PA2 at 10 kDa, because they are good models for any in-chain degradation and because they offered a high ratio of chain-end units, which would be an advantage if thermal depolymerization commenced at the chain end. A side benefit was that, being low MW polymers, they had a content of residual monomer which acted as a positive control indicator in the analytical measurements following heating. Industrial high MW polyacrylamides have residual monomer levels of $<0.05\%$, whereas the 1.5 kDa sample PA1 contained 0.4% residual monomer and the 10 kDa sample PA2 contained 0.05% residual monomer.

Heating Conditions Used. Two heating regimens were studied. Both used a temperature and time relevant to the cooking and browning of foods by baking, frying, or grilling, namely, 15 and 30 min at 175 °C. In the first heating regimen, an aqueous solution of polyacrylamide was applied to a filter paper, and this was heated. This may be considered to approximate a low water activity environment as exists on the surface of a crispbread or bread product as it bakes and browns. In the second regimen, the polyacrylamide solution was added directly to cooking oil and heated. It appeared by visual inspection that the small quantity of polyacrylamide added (10 μ L of a 50% solution in water) did disperse and dissolve in the hot oil (5 g). The hot oil was used to mimic frying of, for example, potato products such as French fries and also to mimic the grilling or baking of fatty/oily foods. Frying temperature depends in part on the smoke point of the oil, and a typical frying temperature should be 160–180 °C (19). At lower temperatures, the products absorb more fat. At higher temperatures, the fat deteriorates more quickly. Using a thermocouple revealed that the filter paper and the oil reached within 5 °C of the set oven temperature (175 °C) within less than 5 and 10 min, respectively. Both heating regimens were tested in the absence and presence of the redox-active metal ions Fe(III) and Cu(II). All tests were conducted without the exclusion of atmospheric oxygen.

Results from Heating in a Drying/Dry Matrix. The results from the heating tests of polyacrylamide applied to paper are shown in **Table 1**. There was no detectable acrylamide in the heated blanks. The unheated control samples revealed that the low MW polyacrylamides contained \sim 19–20 μ g of acrylamide per 5000 μ g of polymer (0.4%) for PA1 and 2–3 μ g of acrylamide per 5000 μ g of polymer (0.05%) for PA2. There was no measurable increase in acrylamide after the polymer had been heated for 15 min at 175 °C and, in fact, the residual monomer level in PA1 fell by about half on heating. The

Table 1. Test Results (Micrograms of Acrylamide Found) from Polyacrylamides Heated on Paper^a

	heated blank, 15 min at 175 °C	unheated control	heated test, 15 min at 175 °C	unheated control	heated test, 15 min at 175 °C
test material	10 μ L of H ₂ O	5000 μ g of PA1	5000 μ g of PA1	5000 μ g of PA2	5000 μ g of PA2
filter paper	<2, <2	19.7, 19.3	9.4, 10.9	2.4, 3.3	2.2, 2.5
paper with added Fe(III)	<2, <2	19.6, 19.0	4.5, 5.0	<2, <2	<2, <2
paper with added Cu(II)	<2, <2	20.3, 18.9	10.9, 7.9	<2, <2	<2, <2

^a Results for duplicate tests reported. PA1, polyacrylamide, MW 1.5 kDa (50% solution, 10 μ L added); PA2, polyacrylamide, MW 10 kDa (50% solution, 10 μ L added).

Table 2. Test Results (Micrograms of Acrylamide Found) from Polyacrylamides Heated in Cooking Oil^a

	heated blank, 30 min at 175 °C	unheated control	heated test, 30 min at 175 °C	unheated control	heated test, 30 min at 175 °C
test material	10 μ L of H ₂ O	5000 μ g of PA1	5000 μ g of PA1	5000 μ g of PA2	5000 μ g of PA2
cooking oil	<2, <2	16.4, 11.8 ^b	7.4, 7.0	2.7, 2.7	2.4, 2.5
oil with added Fe(III)	<2, <2	17.2, 17.5	3.1, 4.7	<2, <2	<2, <2
oil with added Cu(II)	<2, <2	22.1, 16.8	2.8, 4.7	<2, <2	<2, <2

^a Results for duplicate tests reported. PA 1, polyacrylamide, MW 1.5 kDa (50% solution, 10 μ L added); PA 2, polyacrylamide, MW 10 kDa (50% solution, 10 μ L added).

^b Value of 11.8 is considered to be an outlier.

presence of Fe(III), but not Cu(II), in the paper caused a further reduction to about one-fourth of the starting residual monomer content in PA1. The level of acrylamide in PA2 also did not rise, but fell, although the magnitude of the fall cannot be estimated because the levels fell on heating to below the level of quantitation. It can be concluded that heating in a drying/dry environment on filter paper gave no evidence of depolymerization to form acrylamide and that residual levels actually fell due to reaction and/or evaporative losses.

Results from Heating in a Fatty Matrix. The results from the heating tests of polyacrylamide in cooking oil are shown in **Table 2**. There was no detectable acrylamide in the heated blanks. The residual levels of acrylamide in the unheated polymer control were \sim 16–22 μ g/5000 μ g (0.4%) for PA1 and \sim 3 μ g/5000 μ g (0.06%) for PA2. As was found in the tests on filter paper, heating in oil gave no evidence of depolymerization to form acrylamide. Expressed in concentration units, the polyacrylamides were tested at 1000 mg/kg in the heated oil, and no additional acrylamide monomer was detected (<0.4 mg/kg). Monomer levels actually decreased by more than half for PA1 heated in oil and reduced by more than three-fourths for PA1 heated in oil containing added Fe(III) or Cu(II). As was the case with heating on paper, the level of acrylamide in PA2 also did not rise, but fell, on heating in oil, but the extent of the reduction cannot be estimated because the levels fell to below the level of quantitation. Because the samples were heated in sealed vials, making evaporative loss impossible, these reductions in the residual acrylamide monomer content can be attributed to reaction of the monomer in the heated oil.

This work shows that when acrylamide is detected in heated foods, it is likely to come from sources other than depolymerization of polyacrylamides.

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