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Acrylamide in Foods: Occurrence, Sources, and Modeling

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Acrylamide in food products—chiefly in commercially available potato chips, potato fries, cereals, and bread—was determined by liquid chromatography−tandem mass spectrometry (LC-MS/MS). Samples were homogenized with water/dichloromethane, centrifuged, and filtered through a 5 kDa filter. The filtrate was cleaned up on mixed mode, anion and cation exchange (Oasis MAX and MCX) and carbon (Envirocarb) cartridges. Analysis was done by isotope dilution ([D_3]- or [$^{13}C_3$]acrylamide) electrospray LC-MS/MS using a 2 × 150 mm (or 2 × 100 mm) Thermo HyperCarb column eluted with 1 mM ammonium formate in 15% (or 10% for the 2 × 100 mm column) methanol. Thirty samples of foods were analyzed. Concentrations of acrylamide varied from 14 ng/g (bread) to 3700 ng/g (potato chips). Acrylamide was formed during model reactions involving heating of mixtures of amino acids and glucose in ratios similar to those found in potatoes. In model reactions between amino acids and glucose, asparagine was found to be the main precursor of acrylamide. Thus, in the reaction between nitrogen-15 (amido)-labeled asparagine and glucose, corresponding ^{15}N-labeled acrylamide was formed. The yield of the model reaction is ∼0.1%.

KEYWORDS: Acrylamide; model; glucose; asparagine; Maillard reaction; LC-MS/MS

INTRODUCTION

A recent announcement of the discovery of acrylamide in foods for human consumption (*1*, *2*) prompted us to investigate the levels and sources of acrylamide in the Canadian food supply. Acrylamide is a rodent carcinogen and a human neurotoxin and is classified as a probable human carcinogen (*3*). After receiving a brief synopsis of a method developed in Sweden, we independently developed an LC-MS/MS method based on the same principle. Our method incorporates more purification steps than Rosen's procedure (*1*) and thus might be useful for confirmation of acrylamide by detection of its bromo derivative (*4*) as a relatively clean extract is obtained. Initially we analyzed samples either suspected to be high in acrylamide (potato chips/crisps/French fries) or important from a nutritional standpoint (cereals, bread). For some preliminary experiments, we used [D_3]-labeled acrylamide. We switched to using [$^{13}C_3$]acrylamide as soon as it became available to us.

At this time, nothing is known about the sources of acrylamide in foods (see Note Added in Proof). The data published so far indicate that a temperature >100 °C is required for its formation. We tested two formation hypotheses (**Figure 1**). First, acrylamide could be produced from oils and nitrogen-containing compounds present in foods. The most plausible scheme would include the formation of acrolein from the thermal degradation of glycerol (*5*), oxidation of acrolein to acrylic acid, and finally reaction of acrylic acid with ammonia—which potentially could be generated by pyrolysis of nitrogen-containing compounds—leading to the formation of acrylamide. The second hypothesis is that acrylamide could be formed alone, by rearrangement, from nitrogen-containing compounds already present in foods. For modeling studies, we used data on nutrient concentrations in potato tuber from Yang (*6*). Initially we used a mixture of the following five major amino acids present in potatoes (values in parentheses are in micromoles per gram of dry tuber): asparagine (64.2), aspartic acid (18.1), glutamine (16.1), glutamic acid (22), valine (42.2); lysine (3.23), which is present in relatively minor amounts but had been shown to be the most active amino acid in the Maillard reaction (*7*), was also included. On the basis of Yang's data, the concentration of glucose (as equivalent of reducing sugars) was calculated to be 82.3 μmol/g of dry tuber. Later our model was simplified and consisted only of asparagine and glucose. To our knowledge, this is the first time this model system was evaluated for the formation of acrylamide (see Note Added in Proof). There are, however, data on the formation of other compounds in the reaction of asparagine and glucose (*8*, *9*). In this paper, we are reporting initial results of our investigation of the formation and sources of acrylamide in foods.

MATERIALS AND METHODS

Chemicals. Dichloromethane (pesticide grade) and methanol (HPLC grade) were obtained from EM Science (Gibbstown, NJ). Water was obtained from a purification system (Millipore, Milli-Q Gradient A10). All amino acids were of analytical grade. Paraffin oil (heavy grade) was obtained from BDH and acrylamide, 99+%, from Aldrich (catalog

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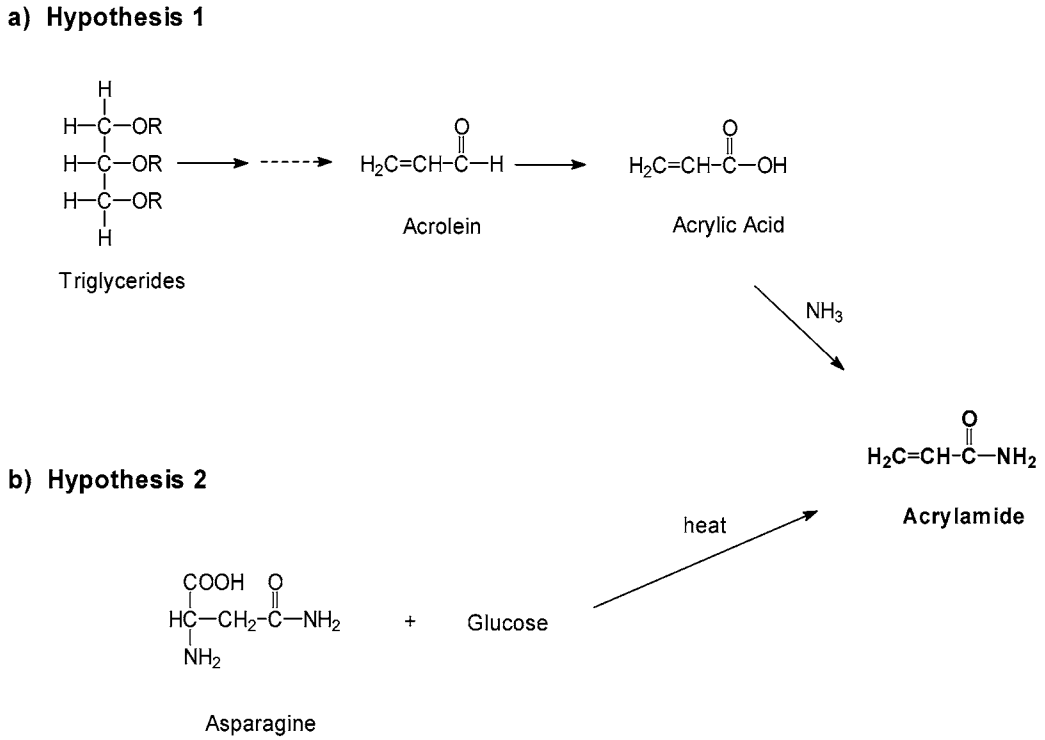

Figure 1. Possible routes of formation of acrylamide.

numbers in parentheses) (14,866-0). Labeled standards [D_3]acrylamide, 99% (DLM-821−1), [$^{13}C_3$]acrylamide, 99% (CLM-813), and asparagine-^{15}N-amide hydrate, 98+% (NLM-120), were from Cambridge Isotope Laboratories (Andover, MA). All stock acrylamide solutions (400 and 250 μg/mL) and calibration solutions were prepared in water.

Foods. All food samples used in this study were purchased locally and stored at 4 °C if required. Olive oil was of extra virgin grade.

Equipment/materials used were as follows: high-pressure liquid chromatograph, model 1100 consisting of an autosampler, a binary pump, a degasser, and a column oven (Agilent, Palo Alto, CA); triple-quadrupole tandem mass spectrometer, Quattro-Ultima (Micromass Inc., Manchester, U.K.); data system, MassLynx version 3.5 (Micromass); analytical column, 2 mm i.d. × 150 mm, 5 μm, Hypercarb (3500-047) with Hypercarb guard column (35007-01210) (Thermo Hypersil-Keystone, Bellefonte, PA); thermostated oven, for example, Star 3600 CX (Varian, Palo Alto, CA), M01490A (Lindberg/Blue M, Asheville, NC); toaster oven TRO 355 TY5 (Black & Decker, Middletown, NY); food processor, Blend Master (Proctor-Silex, Picton, Canada); electric frying pan, 6623 (Toastess, Markham, Canada); homogenizer, Polytron PCU 11 (Kinematica, Littau-Lucerne, Switzerland); centrifuge tubes, FEP, 50 mL, 3114-0050 (Nalgene, Rochester, NY); shaker, horizontal (Eberbach, Ann Arbor, MI); centrifuge, fixed angle rotor, RC-2B (Sorvall, Asheville, NC); centrifuge, swinging bucket rotor, RC-3B (Sorvall); centrifuge filter, 15 mL, 5 kDa cutoff, Centricon Plus-20, UFC2BCC08 (Millipore, Bedford, MA); ultrafiltration membrane, 500 Da cutoff, YC05 (Millipore); mixed mode anion exchange cartridge, 3 mL, 60 mg, Oasis MAX, 186000368 (Waters, Milford, MA); mixed mode cation exchange cartridge, 3 mL, 60 mg, Oasis MCX, 186000253 (Waters); carbon cartridge, 6 mL, 250 mg, Envirocarb 57092 (Supelco, Oakville, Canada); multimode cartridge (C18, cation, anion exchange) 3 mL, 300 mg, 9040030B (IST, Hengoed Mid Glamorgan, U.K.).

Liquid chromatograph mass spectrometer operating conditions (MS/MS mode): mobile phase, 15% methanol in 1 mM aqueous ammonium formate (isocratic); flow rate, 0.175 mL/min; injection volume, 5−10 μL; column temperature, 28 °C; autosampler temperature, 10 °C; ionization mode, positive ion electrospray; desolvation gas temperature, 250 °C; source temperature, 120 ° C; desolvation gas flow, 525 L/h; cone gas flow, 50 L/h; collision gas pressure, 2.6 × 10^{-3} mbar (argon); resolution settings, ∼80% valley separation for both quadrupoles; ion energies, 1.0 V for both quadrupoles; precursor ion → product ion transitions in multiple reaction monitoring (MRM), *m*/*z* 75 → 58 (collision energy = 11 eV); *m*/*z* 72 → 55 (11 eV); *m*/*z* 72 → 54 (11 eV); *m*/*z* 72 → 44 (14) eV; *m*/*z* 72 → 27 (16 eV); cone voltage, 34 V for all MRM transitions, dwell time for each MRM transition, 0.3 s; mass span, 0.1 Da; interchannel delay, 0.05−0.1 s.

Typical Food Sample Preparation and Extraction. The sample was ground, if necessary, in a blender. A subsample (16 g) was homogenized (Polytron) with 80 mL of water in a 150 mL beaker. [Some samples (4 g) were directly homogenized in a centrifuge tube.] The amount of sample might need to be decreased (when high levels of interferences are present, e.g., in potato chips) or increased (when a lower detection limit is required, e.g., for bread). The homogenate (24 g) was transferred to a 50 mL centrifuge tube, and 16 μL of 250 μg/mL (some samples were spiked at a lower level) isotopically labeled acrylamide spiking solution and 10 mL of dichloromethane were added. The mixture was shaken at high speed on a horizontal shaker for 15 min and centrifuged at 15000 rpm (∼24000*g*) in an RC-2B centrifuge for 2 h at 4 °C. The top (water) centrifugate layer (∼10 mL) was promptly transferred to a 5 kDa centrifuge filter and centrifuged at 3500 rpm (∼4000*g*) in an RC-3B centrifuge at 4 °C for 4 h or longer if necessary.

Laboratory Frying Experiments. Medium white Russet potatoes were peeled, sliced into pieces (approximately 3 mm × 30 mm × 40 mm), and fried in batches of ∼100 g in 200 mL of preheated oil in an electric frying pan at 175 °C for 10 or 15 min. Samples fried for 10 min in paraffin or corn oil were spiked during frying with 0.4 g of ammonium carbonate. Samples fried for 15 min in olive or corn oil were spiked before frying with a mixture of 5 g of rosemary herb homogenized in 15 mL of oil.

Typical Food Sample Cleanup. Filtrate from the RC-3B centrifuge (5 mL) was passed through an Oasis MAX cartridge connected in tandem to an Oasis MCX cartridge, and the eluate was collected. The Envirocarb cartridge was conditioned with 5 mL of methanol followed by 2 × 5 mL of water. The eluate from the MAX/MCX tandem was loaded onto an Envirocarb cartridge, the first 1 mL of eluate was discarded, and the remaining portion was collected (fraction 1). The cartridge was washed with 1 mL of water into a separate vial (fraction 2) and again with 1.5 mL of 10% methanol in water into a separate vial (fraction 3). Fraction 1, 2, or 3 was analyzed by LC-MS/MS. In some experiments, fractions 1 and 2 were combined together with the first 1 mL of eluate from the Envirocarb cartridge.

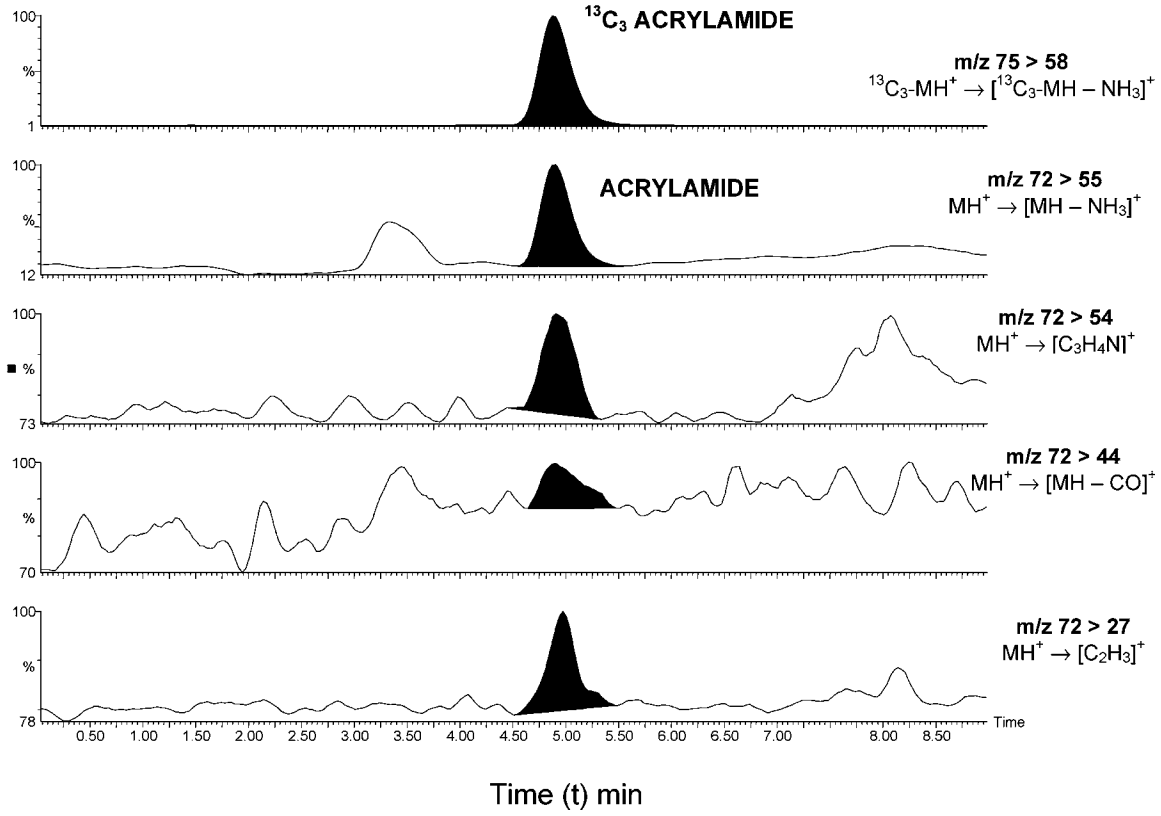

Figure 2. MRM chromatograms of a mixture of 10 ng/mL acrylamide and 50 ng/mL of [$^{13}C_3$]acrylamide standards ($^{13}C_3$-MH$^+$ represents the protonated molecule of [$^{13}C_3$]acrylamide).

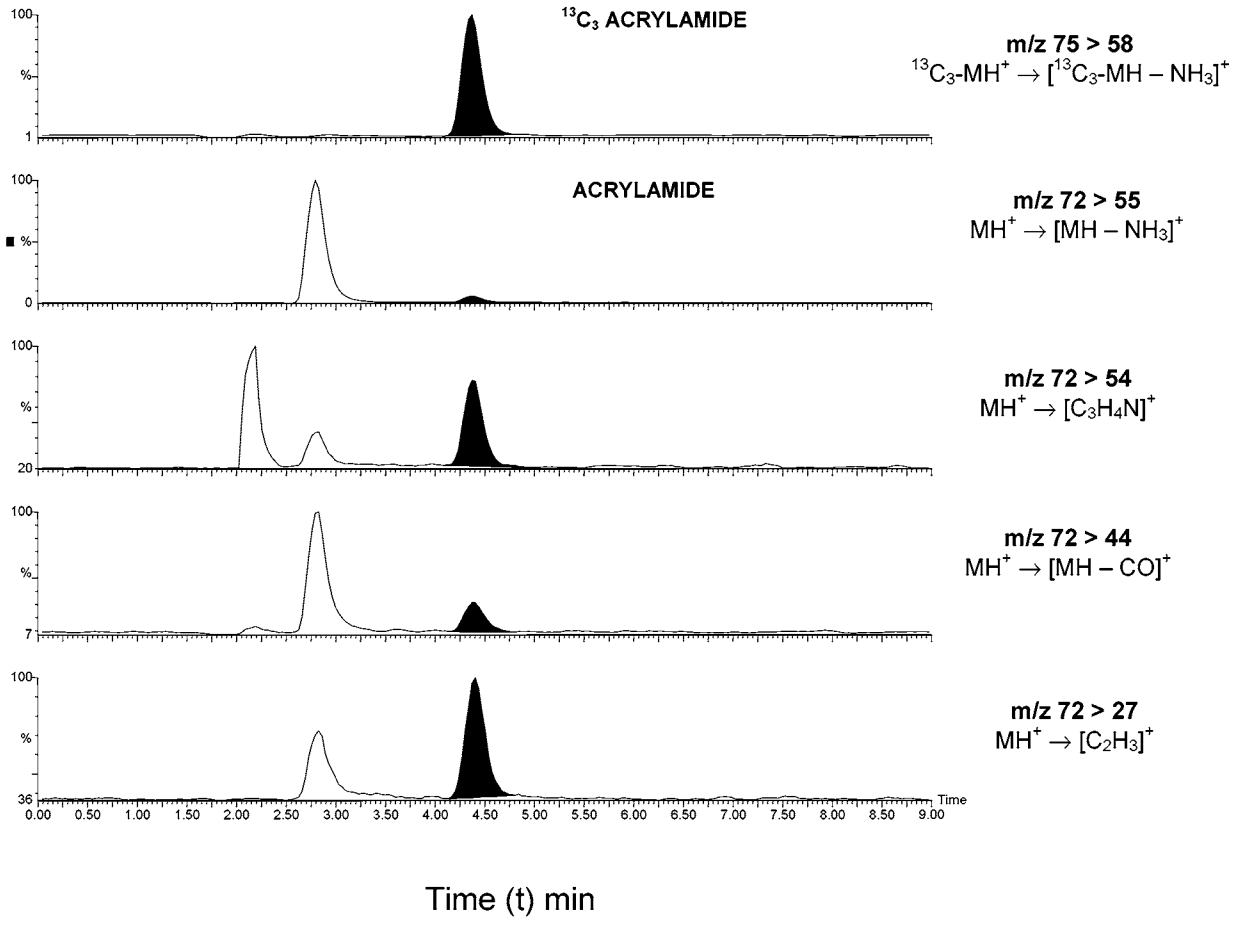

Figure 3. MRM chromatograms of a French fry sample (concentration of acrylamide in the sample was 1900 ng/g).

Model Reaction Condition: Mixture of Six Amino Acids. Amino acids equivalent (*6*) to those contained in 100 g of dry potato tuber [asparagine hydrate (964 mg), aspartic acid (241 mg), glutamine (235 mg), glutamic acid (324 mg), valine (494 mg), and lysine (47 mg)] were mixed together in a mortar. Aliquots of 232 mg of the above amino acid mixture—equivalent to 10 g of dry tuber—were mixed with glucose (150 mg, equivalent to 10 g of dry tuber) and 1 mL of water in a 50 mL tube, placed in an oven, and heated at 175 °C until all water had evaporated (~15 min) and then for another 10 min. Experiments at 120 and 140 °C were done using 116 mg of the amino acid mixture and 75 mg of glucose.

Model Reaction Conditions. *(a) Asparagine and Glucose.* Asparagine hydrate (1.25 g) and glucose (1.50 g) were mixed together in a mortar. Aliquots of 275 mg of the mixture (amino acid/glucose molar ratio 1:1) were heated in an oven in a 50 mL tube (covered with Teflon tape with a pinhole) at temperatures of 155, 165, 175, and 185 °C, for 10, 20, or 30 min.

(b) Free Amino Acids in Pork Meat. A mixture of 16 amino acids [amino acid composition (mg/g of meat, wet weight): Ala (0.28), Arg (1.8), Asp (0.04), Glu (0.13), Gly (0.1), His (0.22), Ile (0.05), Leu (0.08), Lys (0.09), Met (0.07), Phe (0.05), Pro (0.20), Ser (0.07), Thr (0.47), Tyr (0.07), and Val (0.07)] was prepared according to the method of Pais (*10*), and 219 mg of this amino acid mixture was mixed in a mortar with glucose (450 mg) (amino acid/glucose molar ratio 0.63:1). Aliquots of 223 mg of the mixture were heated in an oven in a 50 mL tube at 165 or 175 °C for 10 min.

(c) Asparagine and Glucose in Water in a Sealed Tube. Asparagine hydrate (125 mg) mixed with glucose (150 mg) (amino acid/glucose molar ratio 1:1) and 0.5 mL of water were placed in a 15 mL custom-made high-pressure glass tube fitted with a Teflon stopcock and heated in an oven at 175 °C for 10 min (the tube was preheated to 175 °C over 7 min).

(d) Asparagine-^{15}N-amide and Glucose. Asparagine-amide-^{15}N hydrate (31.25 mg) was mixed with glucose (37.5 mg) (amino acid/glucose molar ratio 1:1) and 0.2 mL of water in a 50 mL tube, placed in oven, and heated at 175 °C until all water had evaporated (~10 min) and then for another 10 min.

Typical Model Reaction Sample Cleanup. The sample was spiked with 16 μL of a 250 μg/mL isotopically labeled acrylamide solution and homogenized with 20 mL of water. The mixture (3 mL) was passed through the three columns connected in series (MAX, MCX, and Envirocarb), and the eluate was collected for LC-MS/MS analysis.

For recovery experiments, 4 g subsamples of a mixture of boiled, mashed Russet potatoes (450 g) and corn oil (50 g) were used and spiked with acrylamide (16−400 μL of 10 μg/mL or 100 μL of 400 μg/mL standard) at 0, 40, 100, 500, 1000, and 10000 ng/g levels. Each level was processed in triplicate and, to simulate between-day variations, on different days. Precision data for the method were obtained by processing six replicates of the French fries sample. For checking the recovery of a model cleanup procedure, 3 mL aliquots of 100 ng/mL or 1000 ng/mL acrylamide standards were processed in triplicate.

RESULTS AND DISCUSSION

MRM chromatograms of a mixture of 10 ppb of acrylamide and 50 ppb of [$^{13}C_3$]acrylamide standards are shown in **Figure 2**. MRM chromatograms of a French fry sample—with a 1900 ng/g concentration of acrylamide—are shown in **Figure 3**. An ion transition *m*/*z* 72 → 55 was used for quantification of native acrylamide, whereas an ion transition *m*/*z* 75 → 58 was used for the isotopically labeled (either D_3 or $^{13}C_3$) acrylamide. The early eluting interference present in channel *m*/*z* 72 → 55 was not removed by filtration through an acetylated cellulose membrane (Millipore) with a 500 Da cutoff. The alternative ionization mode, atmospheric pressure chemical ionization (APCI), resulted in lower sensitivity and did not suppress this early eluting interference either. Initially we used a 100 mm long analytical column, but switching to a longer 150 mm column allowed us to sufficiently separate the interference from the acrylamide peak to afford reliable quantification for most samples. Very recently, we removed this interfering peak by passing our final extract (from the reaction of 16 amino acids and glucose at 175 °C) through an Isolute Multimode column. Using a criterion of a signal-to-noise ratio of 3:1 (peak-to-peak noise definition) at the *m*/*z* 72 → 55 transition, the limit of detection was calculated as ~6 pg of standard injected on-column. The relative standard deviation (RSD) for repeated injection (within-run precision) of the French fries sample containing acrylamide at the 1900 ng/g level was 4.4% (n = 6). An RSD lower than 6% was obtained when six replicates of French fries (with an average concentration of 425 ng/g) were analyzed. For samples (n = 3) of a mixture of boiled and mashed potatoes and corn oil spiked at 0, 40, 100, 500, 1000, and 10000

Table 1. Concentration of Acrylamide in Commercially Available Foods

variety	source	value[a] (ng/g)	% diff of duplicate[b]
potato chips	manufacturer 1	730[c]	10
	manufacturer 2	1500	0.83
	manufacturer 3	3700	7.6
	manufacturer 4	550	9.4
	manufacturer 5	530	
	median	730	
French fries	vendor 1	610	1.4
	vendor 2	1300	26
	vendor 3	620	24
	vendor 4	1900	
	vendor 5	320	
	vendor 6	200	
	median	615	
cereals	rice	100	
	wheat	120	
	oatmeal	170	
bread	100% whole wheat	30	3.7
	light rye	17	
	light rye crust	47	
	toasted light rye	28	
	French bread	16	
	French bread crust	19	
	toasted French bread	290	
	cheese bread	28	
	multigrain	15	
	muesli bagel	14	
	pita, whole wheat	43	
other	roasted buckwheat	35	
	roasted whole coffee "Java"	64	
	roasted almonds	260	
	roasted sunflower seeds	66	
	roasted soy beans	25	

[a] Average values for samples run in duplicate. [b] Separate subsamples. [c] Quantified by standard addition method.

Table 2. Effects of Oil Type, Time, and Additives on Yield of Acrylamide from Potato Slices Fried at 175 °C

oil (type)	additive	time (min)	acrylamide (ng/g)
corn		10	1900
paraffin		10	2200
paraffin	ammonium carbonate	10	1800
corn	ammonium carbonate	10	2250
corn		15	3500
olive		15	5600
olive	rosemary	15	4200
corn	rosemary	15	2500

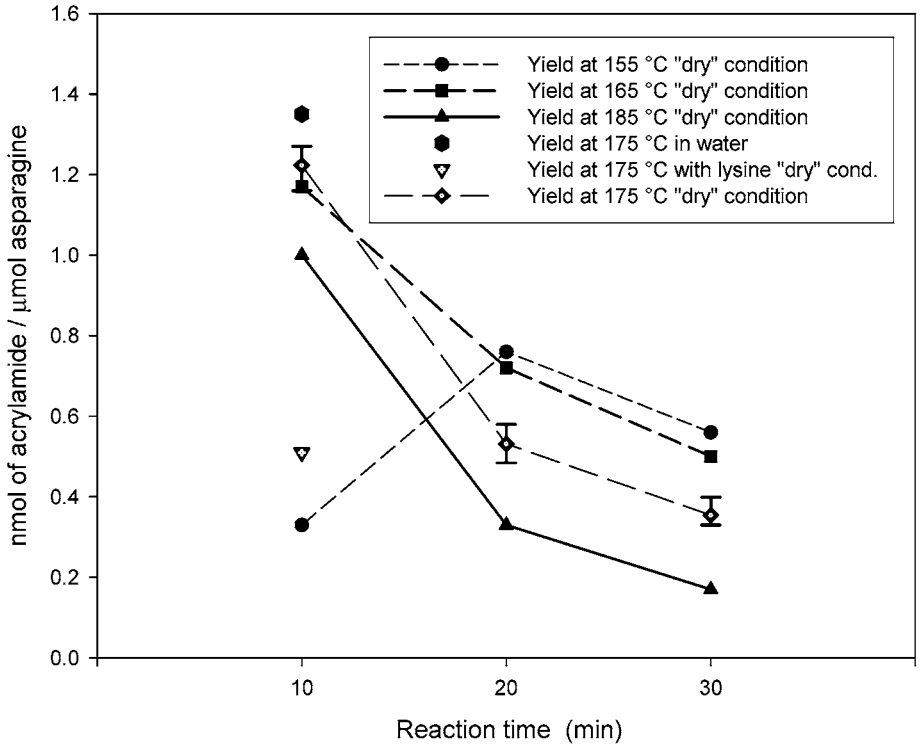

Figure 4. Yield of acrylamide versus temperature and time, glucose/asparagine (molar ratio 1:1) model system.

ng/g levels, the respective concentrations found (ng/g) and % RSD were as follows: nd; 34, RSD 4.4%; 91, RSD 6.7%; 500, RSD 0.7%; 990, RSD 3.6%; 10000, RSD 1%. When acrylamide standards (100 or 1000 ng/mL) were subjected ($n = 3$) to a model cleanup procedure, the respective concentrations found (ng/mL) and % RSD were as follows: 105, RSD 3.2%; 1050, RSD 2%. The five-point calibration curve (concentration of native acrylamide = 10, 25, 50, 100, and 500 ng/mL; concentration of [$^{13}C_3$]acrylamide = 50 ng/mL) was linear with $r^2 = 0.9997$. The 1000 ng/mL standard of acrylamide in water appears to be stable in a clear vial for at least 3 days, but it completely decomposed after 2 months at 22 °C under standard laboratory illumination (fluorescent tubes). We observed a 5% reduction in the response of a 250 μg/mL [$^{13}C_3$]acrylamide stock standard stored for 4 months at 4 °C in the dark.

The levels of acrylamide found in the commercially available potato chips, French fries, cereals, and bread are similar to those found in Sweden (*11*). For example, the ranges of acrylamide (ng/g) in potato chips (330−2300) and in French fries (300−1100) in Sweden are comparable to our data for these matrices, 530−3700 and 200−1900, respectively. In addition to foods of the type analyzed in Sweden, we analyzed a few other food samples that were subjected to roasting and found that all of them contained acrylamide (**Table 1**). The analysis of food samples that were subjected to roasting or baking continues. Our data show striking 6−8-fold differences in the concentration of acrylamide in different varieties of potato products—both chips and fries. One commercial potato chip sample processed with olive oil and rosemary herb had the lowest acrylamide concentration in this food group. Tests in which potato slices prepared in the laboratory were fried in our laboratory in olive or corn oil with or without rosemary herb showed that addition of rosemary reduced the formation of acrylamide by ∼25%, but samples fried in olive oil had a higher concentration of acrylamide as compared to those fried in corn oil (**Table 2**). **Table 2** also summarizes the data obtained during the testing of our first hypothesis (see **Figure 1**) on the formation of acrylamide. Potato samples fried in corn oil and paraffin oil (hydrocarbon-type oil, devoid of triglycerides and thus of acrolein) showed very similar levels of acrylamide. Addition of ammonium carbonate, which decomposes to ammonia gas during frying, only negligibly altered levels of acrylamide when potatoes were fried in these two oils. Thus, it appears that acrylamide is not principally formed from precursors (especially acrolein) present in the oil itself. There was, however, an indication that the type of oil might influence the rate of formation of acrylamide (e.g., olive oil versus corn oil). Also, an increase in frying time (for corn oil) resulted in higher amounts of acrylamide.

We then began testing the second hypothesis that implied the formation of acrylamide from compounds intrinsically present in foods. Initial published data and our results indicated that potatoes are the food that produced the highest amount of acrylamide on frying. We therefore used the composition of potato as a model. We started by investigating the Maillard (browning) reaction between amino acids and glucose. Initially, to simulate baking conditions, six amino acids were heated with glucose in a very small volume of water, which was allowed to evaporate naturally. Later we found (data not shown) that similar levels of acrylamide were produced when only asparagine hydrate and glucose were used. We then continued with the latter scheme as it simplified our experimental procedure.

In model reactions consisting of a mixture of six amino acids, the yield of acrylamide at 175 °C was 3300 ng of acrylamide per quantity of these amino acids (∼23 mg) present in 1 g of dry potato or 0.73 nmol of acrylamide/μmol of asparagine. These values are similar to the results obtained when potato slices were fried in our laboratory at the same temperature. Acrylamide was not detected in a control reaction that lacked glucose. Acrylamide was also not detected in samples of amino acids and glucose heated similarly, but at 120 or 140 °C. However, all samples containing amino acids and glucose exhibited signs of Maillard reaction and produced 5−20 mL of brown brittle foam.

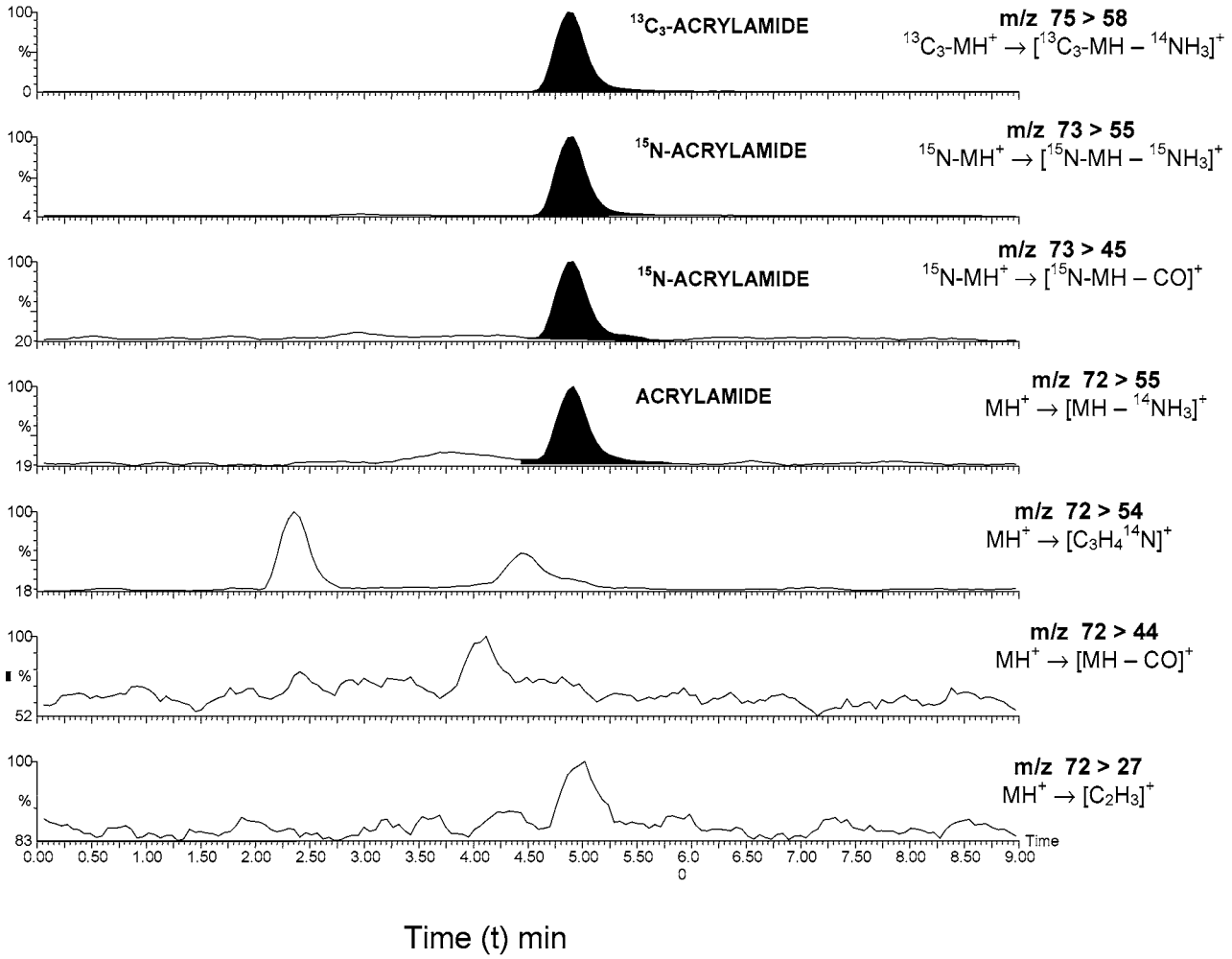

Figure 5. MRM chromatograms of reaction products between asparagine-^{15}N-amide hydrate and glucose at 175 °C.

When a mixture of amino acids resembling the free amino acid composition of pork (asparagine was not present in this mixture) was heated with glucose at 165 or 175 °C, only traces of acrylamide were found in the sample heated at 175 °C. The yield was 4 pmol/μmol of amino acid, which is an ~400-fold reduction when compared with asparagine. Data obtained in this model reaction correspond well with findings of low acrylamide content in fried meat products. In model reaction conditions with amino acids, acrylamide was not detected when asparagine, aspartic acid, glutamine, glutamic acid, valine, or lysine was heated to 120 °C with glucose. Only traces of acrylamide were detected when aspartic acid, glutamine, or lysine was heated at 175 °C with glucose.

When the molar ratio of asparagine to glucose was varied between 0.25 and 4, and the samples were heated at 175 °C for 10 min, the highest yield of acrylamide was obtained at molar ratios of 0.5 to 1:1. At asparagine/glucose ratios of 0.25, 0.5, 1, 2, and 4, the corresponding yields of acrylamide were 1.03, 1.24, 1.22, 0.74, and 0.45 nmol/μmol. The yield of acrylamide from asparagine and glucose versus temperature and time is shown in **Figure 4**. The highest yield was obtained by heating for 10 min at 175 °C (experiments at this temperature were done in triplicate). The level of acrylamide diminished with time except for the reaction carried out at 155 °C, for which the maximum yield was reached at 20 min. When a mixture of asparagine, lysine, and glucose (molar ratio 1:1:2) was heated at 175 °C for 10 min, the yield of acrylamide was only 40% of the value obtained when lysine was not present. The high activity of lysine in the Maillard reaction, attributed to the reactivity of its free amino group, is probably responsible for diminishing the yield of acrylamide. To test the influence of water activity on the yield of acrylamide from asparagine and glucose, we heated these substrates in water (0.5 mL) at 175 °C in a sealed glass tube for 10 min. A yield of 1.35 nmol/μmol was obtained, which was close to the results obtained by using dry heating conditions (**Figure 4**).

Heating of asparagine-^{15}N-amide hydrate and glucose at 175 °C produced a compound having mass fragmentation and retention behavior consistent with those of [^{15}N]acrylamide. The yield of 1.64 nmol/μmol reactant was similar to that obtained with unlabeled asparagine. The 2% yield of native acrylamide (as compared to yield of labeled product) corresponds very well to the isotopic enrichment of the starting material, which had a manufacturer's declared isotopic purity of 98.3% (i.e., the amount of unlabeled asparagine was 1.7%) Therefore, it appears that only the amido nitrogen of asparagine is being incorporated into acrylamide in this reaction. The MRM chromatograms are shown on **Figure 5**. It thus appears that acrylamide is formed by deamination and decarboxylation of asparagine and formation of a C−C double bond. The exact nature of the reaction pathway is currently under investigation, but it likely involves reaction of asparagine with a carbonyl moiety followed by rearrangement(s) of an intermediate.

We have shown that asparagine, when heated with glucose, forms acrylamide with a yield of ~0.1%. We developed a model system that allowed for facile testing of variables involved in the formation of acrylamide. The data obtained through the use of a model system correspond well to data generated so far from

food samples. It is likely that wide variations of acrylamide concentration in foods are, at least partially, caused by different levels of precursors of acrylamide in various batches of raw materials. For example, it is known that levels of asparagine and sugars fluctuate widely in raw potato tubers (*12*, *13*). Moreover, the fact that fried potato products are the food commodity with highest (detected to date) amounts of acrylamide might be related to the relatively high concentration of free asparagine in potatoes. We are currently employing our model system in the investigation of various additives with the aim of reducing levels of acrylamide in foods.

NOTE ADDED IN PROOF

While the manuscript was under review, additional work on the elucidation of sources of acrylamide was done (data not shown). In a reaction of $^{13}C_6$-labeled glucose with asparagine, there is no measurable incorporation of ^{13}C into acrylamide. The amino acid analysis of our aspartic acid and glutamine standards revealed that aspartic acid contained 0.3% asparagine, whereas glutamine contained 0.8% cysteine. The relative yield of acrylamide ("dry" heating in a 50 mL tube) from an asparagine hydrate/sugar system (relative yield for glucose is 1) was 0.48 for sucrose and 1.8 for fructose. The yields of acrylamide from asparagine hydrate and octanal, 2-octanone, and 2,3-butanedione (0.83 mmol each) heated at 175 °C (sealed tube) in water (1 mL) were 0.11, 0.007, and 0.52 nmol/μmol, respectively. The corresponding yields of a reaction done without water were 6.4 ($n = 2$), 0.06, and 0.20 nmol/μmol.

Some of the data in this paper were presented (*14*) as a poster and presentation during the 116th Annual AOAC International Meeting, Los Angeles, CA, September 22−26, 2002.

Three publications and one presentation related to the sources and mechanism of the formation of acrylamide appeared while the manuscript was under review (*15*−*18*).

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