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Sources of Variability of Acrylamide Levels in a Cracker Model

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Surveys determining amounts of acrylamide formed as a byproduct of cooking in frequently consumed fried and baked foods have sometimes found variability in the levels, even when comparing items having similar ingredients and cooking procedures. To better understand the sources of variability, the effects of different ingredients on formation and elimination of acrylamide were studied in a model system based on wheat flour and water, that resembled crackers. It was found that $NaHCO_3$ eliminated acrylamide. To a lesser extent, NH_4HCO_3, cysteine, sodium bisulfite, and ascorbate also enhanced elimination. Some ingredients, including citric acid, ferulic acid, and NaCl, were found to decrease the amount of acrylamide produced while having little or no effect on elimination. Asparagine, but not reducing sugar, caused a large increase in acrylamide formation.

KEYWORDS: Acrylamide; GC-MS; wheat; sodium bicarbonate; baking; crackers

INTRODUCTION

Acrylamide is formed during cooking in some frequently consumed foodstuffs (*1*). Acrylamide has been shown to cause cancer in rodents at very high doses and it has been shown to cause nerve damage in people who were exposed to high doses (*2*). A review has appeared recently that discussed the industrial uses of acrylamide, its toxicology, the mechanism of formation of acrylamide in food and its analysis (*3*). The U.S. FDA performed an exploratory survey of acrylamide in U.S. foods (*4*, *5*). Acrylamide was found in many foods, especially in those that were rich in carbohydrate and were cooked at high temperatures. Some foods showed a large variation in results. For example, wheat cereals contained 77−1057 μg/kg acrylamide, and a Muesli from Switzerland had only 11 μg/kg. When 23 bags of potato chips from one manufacturer were analyzed, the levels ranged from 250 to 550 μg/kg. Other countries have done surveys (*6*−*9*) and similarly found variation. The data from these studies suggest that there may be ways to decrease the amount of acrylamide in food by better understanding the processing conditions and ingredients responsible for the variation.

The formation of acrylamide in gingerbread and ways to reduce its content have been reported, and it was found that replacing ammonium bicarbonate with sodium bicarbonate reduced the acrylamide by >60% (*10*). The effects of asparagine, fructose, and baking conditions on acrylamide content in wheat bread have been reported (*11*). Model studies on potatoes, wheat flour, and starch and ways to reduce acrylamide have been reported (*12*). Methods for determining acrylamide formation and elimination have also been reported (*13*).

Methods based on GC−MS and LC−MS have been used to determine acrylamide in foods (*14*). In one study, it was found that sodium bicarbonate significantly reduced the amount of acrylamide found in gingerbread, in contrast to the high amount found when ammonium bicarbonate was used (*10*). Another model system used yeast-leavened bread, containing wheat endosperm flour, dry yeast, salt, and water (*11*). More than 99% of the acrylamide was found in the crust, and adding asparagine increased the acrylamide from 80 to 6000 μg/kg. Elevated temperature (>200 °C) and time increased the acrylamide, too. Model experiments based on potato, wheat flour, and corn starch were performed with a focus on bakery ware (*12*). Ammonium carbonate or bicarbonate enhanced the yield of acrylamide from asparagine and reducing sugars.

Measuring the elimination as well as the formation of acrylamide can provide important information on mechanisms and ways to reduce acrylamide. Biedermann and co-workers (*13*) pioneered a method for measuring the elimination of acrylamide, in which they added D_3-acrylamide to hash brown potatoes before frying and analyzed the cooked product by GC−MS; the mass spectrometer distinguished between acrylamide and the deuterated analogue. Despite the lower asparagine content, wheat was still a good model system to study acrylamide formation and elimination as reported here. Recipes included water as an ingredient and a dough mixing step before baking. Both simplified the addition of the D_3-acrylamide and other test ingredients in a uniform manner. The system utilized a cracker-like product. Crackers have amino acids, proteins, and a high carbohydrate content and they are cooked at high temperatures. They have the potential to make acrylamide, since they contain asparagine and reducing sugars, but it is possible to reduce the amount of acrylamide produced.

MATERIALS AND METHODS

The 1,2,3-$^{13}C_3$-acrylamide (99%), and 2,3,3-D_3-acrylamide (98%) were from Cambridge Isotope Laboratories (Andover, MD). Pastry flour (100% soft wheat, same brand used throughout), soybean oil (two

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samples), canola oil, corn oil and partially hydrogenated soybean oil were obtained from a local grocery store. Solvents were pesticide residue grade; ethyl acetate from EM Science (Gibbstown, NJ), deionized water prepared in house, and remaining solvents from Burdick & Jackson (Muskegon, MI). The other chemicals were from Sigma Aldrich (St. Louis, MO). Chemicals and food ingredients were greater than 98% purity except for δ-tocopherol (90%) and wheat gluten (80% protein). The 3 mL unbuffered ChemElut Hydromatrix (diatomaceous earth) columns were from Varian (Walnut Creek, CA). Maxi-Spin centrifuge filter tubes were 50 mL with 0.45 μm nylon membrane from Alltech (Deerfield, IL).

CAUTION: Acrylamide is a potent neurotoxin in man and animals and may be carcinogenic. Avoid contact and inhalation exposure.

Preparation of Crackers. Recipe 1 consisted of 150 g of pastry flour, 80 mL of deionized water, plus 2 mL of 200 μg/mL D_3-acrylamide in water. For most experiments an additional ingredient was added to the recipe to determine the effect on elimination and net formation of acrylamide. These ingredients and amounts used are listed in Table 1. In one case, $^{13}C_3$-acrylamide was used in place of the deuterated isotope to ascertain if deuterium exchange was significant. Recipe 2 consisted of 150 g of pastry flour, 70 mL of water, 3 g NaCl, 5 g $NaHCO_3$ and 18 g of soybean oil plus 2 mL of 200 μg/mL D_3-acrylamide in water. Additional experiments were performed with asparagine and/or glucose added to Recipe 2.

The water-soluble ingredients were dissolved and the oil-soluble ingredients were mixed with the oil, except for one experiment where MCP dry powder was blended with the flour. The flour was added to the water and the oil (if used) was poured on top. It was mixed for 2.5 min in a stainless steel beaker with a handheld mixer using dough hooks, rested at room temperature 30 min and then kneaded by hand for 1 min. About 100 g of the dough was formed into a sheet 0.32 cm thick using a rolling pin. The thickness was controlled by surrounding the dough with a 20 cm diameter loop of 0.32 cm od stainless steel tubing. The sheet was cut into squares of 35 mm on each side and 16 evenly spaced holes of about 2 mm diameter were made in each to permit some ventilation. Six pieces were individually weighed and then baked by convection heating on a wire grid suspended in a surplus GC oven for 5−45 min at 180 °C (except as noted). Each cracker was removed from the oven at the appropriate time and cooled to room temperature.

Determination of Acrylamide in Crackers by GC−MS. Analysis always commenced immediately after removal from the oven. Each cracker was weighed, ground in a mortar and pestle, and 1 g was added to a centrifuge filter tube followed by 5 mL of water. A 1 g sample of dough was added to a centrifuge tube followed by 5 mL water, and a glass rod was used to form a slurry. When measuring recoveries of the extraction and cleanup procedures, an appropriate amount of D_3-acrylamide was added to the 5 mL extraction water after the sample was baked and ground, instead of being included in the recipe. The mixture was shaken by hand for 15 min. To remove fat, 4 mL of hexane was added, followed by thorough mixing. The tube was centrifuged at 3000 × *g* for 30 min using a fixed angle rotor (IEC, Model HT). The hexane was discarded and 2 mL of the aqueous layer was applied to a Hydromatrix column that had been pre-washed with 10 mL of CH_2Cl_2 and dried. The water became immobilized as a thin layer on the Hydromatrix after about 10 min. The acrylamide was eluted off the column using 30 mL of ethyl acetate. The volume was reduced to 1.0 mL in a graduated centrifuge tube under a stream of nitrogen, while being heated to 60 °C prior to analysis by GC−MS. One μL was injected onto a Varian GC-MS, consisting of a 8200 autosampler, a 7980 injector (SPI with buffer insert, deactivated, 60−230 °C in 1 min), a 3400 GC, 5 m polar deactivated retention gap, 0.32 mm, and a dbWAXETR column (15 m × 0.25 μm × 0.25 mm) from Agilent/J&W (Wilmington, DE). The temperature program was 60−160 °C at 12 °C/min, followed by 160−230 °C at 50 °C/min. A Saturn 4D ion trap mass spectrometer operating in the chemical ionization mode with methane and a mass range of 60−80 was used. The peak areas of the $[M+1]^+$ ions, *m*/*z* 72 and 75, were integrated for acrylamide and D_3-acrylamide, respectively. A standard curve for each run was made at acrylamide concentrations of 20, 100, and 1000 μg/mL in ethyl acetate. D_3-acrylamide concentrations were calculated based on the weights of dough before baking, acrylamide concentrations in crackers were based on the weight after baking.

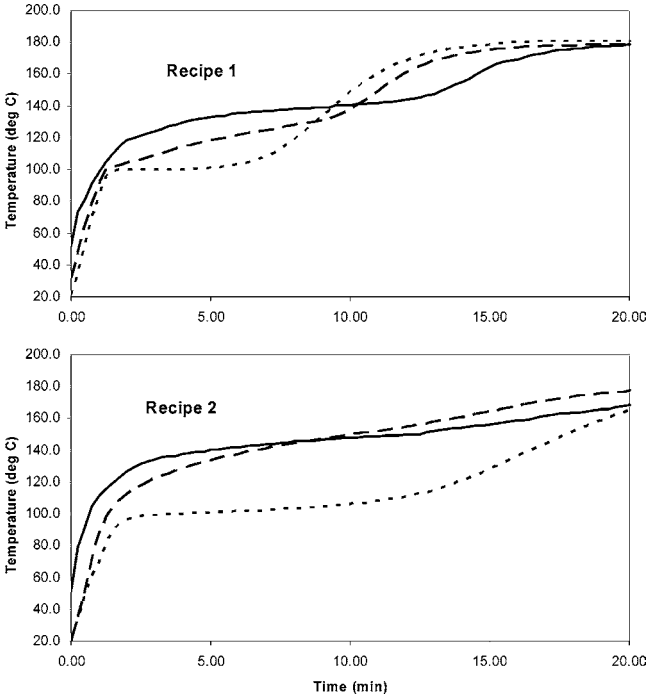

Figure 1. Temperature during baking measured at the cracker top (infrared, solid line) middle (thermocouple, dotted line) and bottom (thermocouple, dashed line).

Dough pH. In a few cases, it was necessary to estimate the pH of the dough. This was done by taking 1 g of dough, forming a slurry with 10 mL water, and measuring the pH (Orion 520A) after about 1 h.

Determination of Cracker Temperatures During Baking. Doughs made from Recipes 1 and 2 were formed into the cracker shape to study temperature at the center as a function of baking time. A 2 × 18 cm wire grid was attached to a Cole-Parmer (Vernon Hills, IL) DuaLogR thermocouple thermometer below two 6 in long Omega (Stamford, CT) MQ series Type-T probes with stainless steel sheath diameters of 0.5 and 0.81 mm. The grid provided support for the dough when the two probes were inserted about 4 mm apart, both near the middle of the thickness, or both near the bottom center, just below the surface. The dough was inserted through a hole in the side of the oven, baked at 180 °C and the temperatures were recorded every 15 s until the samples approached oven temperature. Six samples of a recipe were baked and the resulting 12 measurements were averaged.

An infrared-to-analogue converter module, Omega OSM101, was used to estimate the surface temperature of the crackers. The cracker dough was placed on a wire grid near the middle of the 180 °C oven. The infrared sensor was placed above a hole in the top of the oven and aimed so the dough filled the field of view, and the temperature was recorded at 15 s intervals with the thermocouple thermometer. Three samples of a recipe were baked and the results averaged.

RESULTS AND DISCUSSION

Cracker Model System. The temperature as a function of time is shown in **Figure 1**. Although the model system is a type of cracker, it is not based on commercially prepared crackers. Recipe 1 was simply a mixture of wheat flour and water in a ratio similar to that used for bread (*15*), and the dough had viscoelastic properties making it easy to handle, soft without stickiness. Recipe 2 added three more ingredients that are generally used for bread, sodium chloride, sodium bicarbonate, and oil, approximating typical amounts (*15*), and the water volume was reduced in order to maintain the desired viscoelastic properties. The baking temperature of 180 °C was selected

because it is near the high end of the range normally used for home baking, it produced a relatively high level of acrylamide without excessive losses due to decomposition or evaporation and the crackers baked evenly in a reasonable amount of time. This temperature is also consistent with other workers who found 180 °C to be the optimum for a wheat/water model system when there were no added ingredients (*12*). Baking the dough as a thin cracker was done to promote relatively even baking, with a suitable temperature for acrylamide formation eventually being reached throughout the entire sample. The crackers were generally found, by visual inspection, to have only a very thin region at the surface slightly darker than the evenly browned interior. This is contrasted with, for example, bread making, where there is a significant temperature gradient at the end of the baking time from 100 °C at the center to oven temperature in the crust (*16*). Although the recipes were not necessarily designed to produce foods to be eaten, the samples baked for 10 and 15 min were judged to have the degree of doneness expected from home baked crackers, based on moisture level, texture, color, and aroma. Samples baked for longer times model more closely resembled the overcooked parts of baked wheat products that are dry and hard. The acrylamide levels at longer baking times are best described as indicating the formation potential.

Labeled acrylamide was added to determine the amount eliminated during the baking process, following the example set by Biedermann et al. (*13*) for a model system using hash brown potatoes. When another ingredient was added to a basic recipe the amount was generally chosen arbitrarily in an exploratory effort to discover factors that influence the net amounts of acrylamide found in foods, not necessarily related to ingredient proportions normally used in foods. In some cases, where noted, the amount of an ingredient was chosen to be the molar equivalent of an ingredient used in a separate experiment to make a valid relative comparison.

Recipe 1 was found to provide a relatively inert environment for acrylamide in a food that has the precursors necessary for formation (**Table 1** and **Figure 2a**). After baking times of 10 and 15 min, the D_3-acrylamide was down less than 10% and 20%, respectively, compared to the amounts recovered from dough, while the acrylamide formed was 149 and 581 μg/kg, respectively. It is, therefore, a good model to study added ingredients that can increase the elimination of acrylamide. Finding such ingredients can potentially lead to a better understanding of mechanisms and the ability to make predictions about other food additives that might be useful. In contrast, Recipe 2 eliminated the labeled acrylamide at a faster rate (**Table 1**, **Figure 3**). After baking times of 10 and 15 min, the D_3-acrylamide was down about 91 and 95%, respectively, compared to the amounts recovered from dough. The net amount of acrylamide formed in Recipe 2 at baking times of 10 and 15 min was down about 69 and 87%, respectively, compared with Recipe 1. Clearly, the increased elimination rate in Recipe 2 plays an important role in the reduction of acrylamide, but it is not easy to determine from these data whether there is also a change in the rate of formation. Both recipes have similar amounts of acrylamide precursors in the wheat flour, and the data show that $NaHCO_3$ was the ingredient in Recipe 2 most responsible for the increase in elimination. This can be seen from experiments where each ingredient was individually added to Recipe 1 (**Table 1**). Recipe 2 is, therefore, a good model system to study factors that might increase the net amount of acrylamide, either by accelerating formation or blocking the elimination mechanism.

Although the list of ingredients in **Table 1** implies that only one variable at a time was tested, there were indirect changes caused by some that could not be controlled. During dough formation and baking, flour and water form a cohesive, extensible mass that retains gases evolved, and the viscoelastic properties can be changed by added ingredients that either strengthen or weaken the protein matrix (*17*). Elasticity is related to the formation of disulfide linkages, while noncovalent intermolecular forces contribute to viscosity. Some ingredients can either reduce disulfide linkages (i.e., cysteine) or oxidize sulfhydryls (i.e., ascorbate). Sodium chloride tends to strengthen the gluten network. Lipids form complexes with proteins by association of hydrophobic areas or ionic interaction between protein side chains and ionic groups of phospholipids or fatty acids (*17*). Physical changes in viscoelastic properties can potentially contribute to variations in acrylamide levels. One way this can occur is when changes in the size and number of gas bubbles and the overall shape of the cracker cause changes in the migration of water or steam, which potentially changes the migration of acrylamide precursors and the temperatures of different regions where acrylamide can be formed. Relatively small changes in rates of acrylamide formation or elimination are of little practical value for controlling levels in food. It is clear from the large dataset that changes caused by some of the ingredients exceed those from uncontrolled variables, and it will also be possible to make valid comparisons among sets of samples having similar ingredients.

Method Performance. The Hydromatrix partitioning step was similar to that reported as part of another acrylamide procedure (*18*) and was found to give a 95% recovery. The extraction and cleanup method performance was evaluated by determining the recovery of acrylamide in samples spiked after baking. The overall recovery after baking for both recipes 1 and 2 at four different baking times was 70−100% in crackers spiked with D_3-acrylamide (data not shown). At least six experiments involving the complete procedure from dough preparation through baking and analysis were repeated and values agreed within 20%. The limit of quantitation was 30 μg/kg and the limit of detection was 15 μg/kg. It was found that by comparison with results for Recipe 1 made with $^{13}C_3$-labeled acrylamide that deuterium−hydrogen isotopic exchange was negligible (**Table 1**), in agreement with others (*12*, *13*).

Every set of baked crackers included one sample of raw dough that was also assayed. Although most doughs with recipes 1 and 2 had an added ingredient there was no evidence that D_3-acrylamide (**Table 1**, % recovered at 0 min) was greatly affected by the change during the few minutes that the dough remained at room temperature prior to extraction, except where the additive was cysteine, sodium bisulfite or the highest concentrations of NaOH, where a direct reaction with acrylamide was expected and observed. After excluding those few cases, the recovery of D_3-acrylamide from the doughs ($n = 57$) ranged from 53.1 to 95.1% with an average of 70.7% and a 13.5% relative standard deviation. Recovery from dough represents only one of the 14 data points for each experiment; the uncertainty is reduced somewhat and the interpretation becomes easier when the full data is taken into consideration for making comparisons between samples.

Effect of Temperature. The acrylamide level in food is a function of cooking temperature when other variables are held constant. Formation is generally believed to begin around 120 °C, increasing to about 200 °C, and above that elimination mechanisms begin to predominate (*2*). Two sets of samples were baked at other than 180 °C to confirm that the results are

Table 1. Acrylamide Formation and Elimination in Crackers with Added Ingredients

	net acrylamide formed (μg/kg based on dry weight)						% recovery labeled acrylamide (based on dough weight)						
	time (min)						time (min)						
	5	10	15	20	30	45	0	5	10	15	20	30	45
recipe 1 (n=3)	t	149	581	1870	1850	1630	83.6	78.7	76.9	69.7	42.9	26.5	18.3
recipe 1 ($^{13}C_3$ label)	t	156	606	2040	1670	1480	78.3	83.6	82.6	75.8	47.4	26.2	20.7
recipe 1 (140 °C)	n	n	n	t	153	352	77.8	91.6	77.2	84.0	79.5	67.6	55.6
recipe 1 (220 °C)	184	813	1380	566	n/a	n/a	n/a	83.6	69.7	19.9	3.5	n/a	n/a
					recipe 1 +								
3 g NaCl	n	67.2	335	671	812	686	59.0	93.8	76.5	66.8	42.9	18.6	10.1
7.5 g NaCl	n	61.3	201	886	994	858	67.5	64.5	61.3	54.5	29.0	14.7	7.9
2.5 g $NaHCO_3$	t	75.2	111	277	271	237	53.1	11.2	5.3	3.1	2.8	t	t
5.0 g $NaHCO_3$	n	t	t	52.0	75.9	73.5	59.0	13.5	3.7	2.2	t	t	n
7.5 g $NaHCO_3$	n/a	t	t	t	43.3	54.3	61.1	12.9	4.2	t	t	t	n
8.9 g $KHCO_3$	n	t	48.5	81.2	75.9	68.4	64.3	13.5	3.0	t	t	n	n
5 g NH_4HCO_3	75.5	227	610	965	1920	1940	82.8	73.4	48.3	53.7	51.9	29.2	16.1
7.5 g NH_4HCO_3	t	162	453	661	1430	1300	63.3	56.0	59.7	46.0	42.6	23.1	13.8
5 g $NaHCO_3$+6 g MCP	t	125	259	552	601	523	58.2	51.1	45.7	41.6	29.6	13.1	8.1
1 g NH_4Ac	40.6	285	604	1600	1250	1140	79.0	68.2	74.9	62.4	38.4	15.7	12.5
1 g sodium bisulfite	n	212	1260	1780	1320	966	49.9	33.0	16.5	6.6	3.3	t	t
5.8 g sodium ascorbate	n	168	527	582	322	301	85.9	79.8	62.7	27.7	6.3	3.7	2.8
17.5 g sodium ascorbate	n	77.7	245	178	114	71.1	80.4	75.2	45.4	8.9	2.9	t	t
5 g ascorbic acid	n	38.6	180	478	499	492	78.1	69.4	58.7	49.0	13.9	7.1	5.4
15.5 g ascorbic acid	n	t	70.1	111	141	125	70.8	63.1	44.6	10.1	t	t	t
5 g ferulic acid	n	66.3	218	735	799	824	71.7	66.2	74.4	61.5	49.4	32.4	28.5
2 mL 6N HCl	n	t	89.7	420	536	585	58.7	53.6	64.6	56.6	41.6	24.9	18.7
4 mL 6N HCl	n	n	t	105	139	170	63.9	69.2	63.0	53.1	32.7	18.8	13.4
1.25 g citric acid	n	73.4	348	573	687	705	64.3	61.3	67.4	49.1	40.9	28.2	20.0
2.5 g citric acid	n	t	89.2	243	381	452	54.3	53.5	54.7	53.3	36.3	24.6	19.1
0.19 g NaOH	t	125	628	1480	1780	1520	79.0	93.9	75.7	61.3	39.5	24.5	12.6
0.42 g NaOH	t	225	755	1840	1790	1400	80.5	64.7	53.5	40.0	23.3	13.4	9.4
0.58 g NaOH	t	117	466	760	627	472	53.0	31.3	22.4	12.5	9.3	5.3	3.5
1 g methionine	t	117	510	1160	1210	1100	70.4	69.7	59.4	46.4	19.7	15.6	9.4
1 g asparagine	70.7	1600	7310	20100	23300	22200	86.2	68.1	68.4	51.5	35.5	16.2	13.9
0.5 g cysteine	n	84.2	988	1390	1020	920	52.9	15.9	6.4	2.2	t	t	t
1 g cysteine	n	t	279	1210	998	870	56.8	10.7	2.7	t	n	n	n
0.5 g glucose	34.0	190	761	1530	1450	1120	64.7	78.6	78.2	70.0	42.5	25.0	20.8
1 g fructose	33.7	378	1270	1850	1500	1250	95.1	91.8	82.4	62.4	39.8	24.2	14.4
0.5 g glucose + 0.5 g asparagine	107	1680	4710	11500	9510	8480	67.1	75.7	69.6	61.8	33.4	14.7	9.0
1 g asparagine + 1 g NH_4Ac	412	3480	16400	22500	21400	17300	72.2	73.6	70.7	42.0	20.4	9.1	6.4
1 g gluten	t	120	327	1210	1510	1330	82.7	82.8	87.4	73.8	56.8	31.9	20.3
15 g gluten	t	134	293	680	1110	1160	81.2	69.0	74.7	72.1	53.7	24.5	13.3
15 g casein	49.2	109	324	587	811	800	76.5	76.2	65.1	60.3	44.8	21.9	7.7
5 mL Niaproof	n	293	1030	2620	2610	1840	n/a	69.6	69.1	49.8	31.2	20.3	12.1
18 g silicone oil	t	210	1020	3110	1360	1230	64.7	60.6	53.4	38.0	61.2	16.2	12.4
18 g wheat germ oil	t	331	1320	1980	2240	1970	61.8	55.9	48.1	40.2	29.9	17.7	14.7
18 g corn oil	t	208	1100	1760	1840	1660	62.4	59.7	52.3	42.9	30.0	18.3	12.0
18 g soybean oil (n=3)	t	283	823	1240	1250	1066	62.5	60.8	54.9	39.4	30.0	18.1	11.9
18 g canola oil	t	196	700	867	1020	1190	60.0	64.6	58.6	43.6	36.9	20.3	16.9
18 g olive oil	t	431	1250	1660	1680	1580	63.0	57.9	51.2	38.8	29.5	21.1	13.3
18 part. hyg. soybean	t	362	1680	2090	1880	1740	77.8	71.4	56.9	44.6	35.4	20.1	11.9
10 g vitamin A*	t	196	594	1000	949	972	71.3	64.8	56.6	40.5	28.1	17.8	11.3
36 g vitamin E	t	194	1030	1460	1320	579	66.0	63.5	52.8	35.1	24.4	15.6	9.5
1 g beta-carotene*	n	177	864	1340	1200	1270	61.0	64.0	56.0	40.3	34.5	19.0	13.3
37.2 delta-tocopherol	53.5	469	1470	1490	1230	1120	72.5	49.7	52.9	33.3	24.1	16.0	13.2
4.6 g linoleic	t	219	729	1710	1830	1630	72.4	66.9	61.7	51.8	31.8	22.5	15.3
6 g linolenic	t	158	622	1130	1400	1330	64.6	64.7	61.5	50.9	39.6	25.5	16.8
4.7 g oleic acid	t	228	638	1520	1510	1360	59.6	61.1	60.3	49.9	34.4	22.6	14.3
recipe 2 (n = 2)	t	45.8	74.9	107	171	166	77.5	26.5	6.8	3.6	t	n	n
					recipe 2 +								
1 g NH_4Ac	51.9	82.7	120	159	242	211	76.2	35.1	17.5	6.9	4.8	t	n
0.5 g asp	69.3	186	273	559	631	872	69.6	20.9	8.1	3.5	2.2	t	n
0.5 g glu, 0.5 g asp	139	264	547	888	1050	1050	64.9	19.2	4.6	2.7	t	t	n

In all but one case, where noted, the labeled material was D_3–acrylamide. The baking temperature was 180 °C except where noted. Acrylamide formed at time=0 is not shown because in all cases it was below the detection limit. t, trace, is between 15 and 30 μg/kg; n, not detected, is less than 15 μg/kg; n/a indicates not measured; * dissolved in 18g wheat germ oil.

consistent with those reported by others (**Table 1**). When recipe 1 was heated at 140 °C, the crackers baked for 30 and 45 min were judged to have a degree of doneness similar to recipe 1 crackers baked at 180 °C for 15 and 30 min; the acrylamide levels formed at the comparable times were about 20−25% the amounts at the higher temperature. Recovered amounts of the D_3-acrylamide did not decrease significantly with baking time. When recipe 1 was heated at 220 °C, the acrylamide level both rose and fell earlier, compared to heating at 180 °C, reaching a maximum value at 15 min that was about 75% the maximum

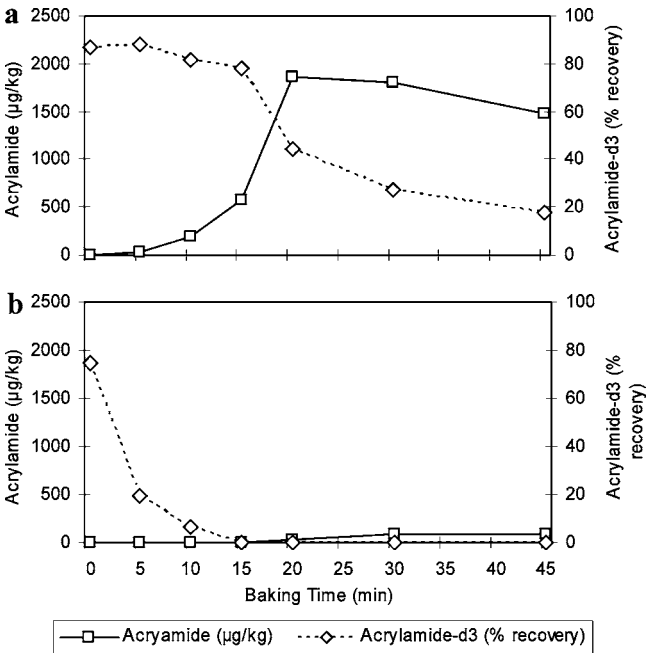

Figure 2. Formation and elimination of acrylamide as a function of baking time at 180 °C using (a) recipe 1 (150 g soft wheat flour, 0.4 mg D_3-acrylamide in 80 mL water) and using (b) recipe 1 with 7.5 g $NaHCO_3$ added to the water.

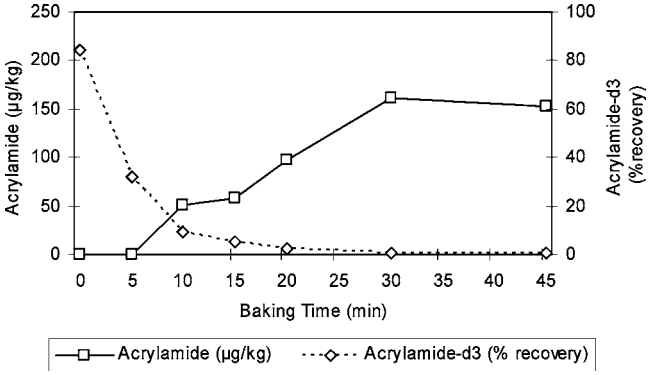

Figure 3. Formation and elimination of acrylamide as a function of baking time at 180 °C using recipe 2 (150 g of pastry flour, 0.4 mg of D_3-acrylamide, 70 mL of water, 3 g NaCl, 5 g $NaHCO_3$ and 18 g of soybean oil).

value after 20 min at 180 °C. The D_3-acrylamide recovery data shows that the increase in elimination at the higher temperature is largely responsible for the decrease in net acrylamide formation.

Experiments were performed to make rough correlations between the temperatures of the crackers during baking and the amounts of acrylamide formed or eliminated. The acrylamide value for a sample was an average of the entire cracker because it was ground to a powder before analysis, but the thermocouples and infrared sensor could only measure relatively small non-representative volumes of a sample. As a compromise, measurements were made in separate experiments at three locations in typical crackers; probes were inserted into the middle of the dough thickness or at the bottom just below the surface, and the upper surface was measured by infrared. The temperature at the middle rose to and stayed at a plateau near 100 °C until moisture decreased sufficiently to permit a gradual rise to the 180 °C oven temperature. The rise to oven temperature was delayed longer in recipe 2 compared to recipe 1, apparently because of the inclusion of sodium bicarbonate in the ingredients (data not shown), but the temperature at the bottom was significantly higher during that time interval. The upper surface of the recipe 1 cracker was cooler than the two internal locations between about 10 to 20 min. Crackers baked with either recipe attained temperatures high enough to form acrylamide at some locations before 5 min, and by 10 min the entire recipe 1 cracker was hot enough.

Bicarbonate Salts. The dramatic changes in elimination and net formation of acrylamide caused by sodium bicarbonate are shown in **Figure 2a,b**, comparing recipe 1 before and after 7.5 g $NaHCO_3$ addition, and the concentration dependence at 2.5, 5.0 and 7.5 g (0.95, 1.9, and 2.8 wt % in the dough) is shown in **Table 1**. All three concentrations had a similar effect on elimination. Net acrylamide increased somewhat as bicarbonate dropped to near 1%, but remained significantly suppressed compared with recipe 1. Potassium bicarbonate, when added at the molar equivalent of 7.5 g $NaHCO_3$ (**Table 1**), had an effect similar to the sodium compound except that net formation appeared to be slightly higher. Adding either 3.0 or 7.5 g NaCl to recipe 2 reduced the acrylamide formation to roughly half the amount in Recipe 1 (**Table 1**). Thus, the effect of sodium bicarbonate is not entirely due to the sodium.

The results with ammonium bicarbonate were quite different from the sodium and potassium salts. Crackers baked with 5.0 or 7.5 g NH_4HCO_3, were compared to those with the same weights (roughly molar equivalents) of the sodium salt (**Table 1**). The elimination with the ammonium bicarbonate was only slightly greater than in the basic recipe 1, and the net formation was about the same as in recipe 1 except for a decrease at 20 min; both of which contrasted sharply with the corresponding comparisons of the sodium and potassium salts with recipe 1. Although this is useful information, the interpretation of the data is again complicated by the change in the temperature curve known to accompany bicarbonate addition. Another variable is the relatively high volatility of the ammonium bicarbonate upon heating compared with the sodium salt.

Two earlier papers reported the effect of carbonate or bicarbonate salts on acrylamide formation in wheat based model systems (*10*, *12*), and both found that ammonium bicarbonate greatly enhanced the amount of acrylamide in recipes containing added sugar. It was noted that both ammonium and added sugar were required to enhance the acrylamide formation mechanism (*12*). In the work reported here, however, the ammonium bicarbonate was tested only in a recipe without added sugar, and that apparently accounts for the lack of an increase in acrylamide relative to the basic wheat/water recipe. This work is in agreement with the others in finding that samples baked with ammonium bicarbonate have more acrylamide than samples baked with the sodium or potassium bicarbonates.

Recipes that use sodium bicarbonate for leavening typically add a leavening acid to increase the conversion to CO_2 and reduce the amount of residual carbonate. An experiment was performed using MCP, an ingredient in fast acting baking powders. Five g sodium bicarbonate and 6 g MCP were both added to recipe 1. The MCP powder was mixed with the flour before being added to the bicarbonate solution in order to minimize the amount of CO_2 released prior to dough formation. The pH of the dough was 7.5. The crackers baked from this recipe remained flat, while crackers baked with just bicarbonate were increased in volume by the trapped CO_2. This indicates MCP converted much of the bicarbonate to CO_2 before baking. The data in **Table 1** shows that MCP greatly reduced the effectiveness of bicarbonate in eliminating acrylamide. This is consistent with the hypothesis that ammonium bicarbonate is

less effective in eliminating acrylamide compared to sodium bicarbonate because it is more volatile.

Sodium Hydroxide. The possibility was initially considered that $NaHCO_3$ eliminated acrylamide in baked crackers by raising the pH. For example, it was reported that, for the reactions between sulfhydryl groups and α,β-unsaturated compounds, the nucleophile is the thiolate anion (*19*) which generally forms at pH 8 and above (the pK_a of the sulfhydryl in free cysteine is 8.3), and the pH of recipe 1 was raised from 6.3 to 8.5 by the addition of 7.5 g sodium bicarbonate. Many other reactions could potentially be influenced by pH changes. The buffering capacity of the flour made it difficult to raise the pH with aqueous buffers, so the pH of the dough was varied by dissolving amounts of solid NaOH in the 80 mL water component of recipe 1. Additions of 0.19 and 0.42 g NaOH raised the pH from 6.3 to 7.4 and 8.3, respectively, and the effect on both elimination and total formation is negligible (**Table 1**). The $NaHCO_3$ elimination mechanism appears to be more than a simple pH effect. Only when the pH was raised to 9.6 and 10.5 by 0.58 and 0.78 g NaOH, respectively, did the pH effect become significant.

Proteins. The addition of protein as gluten or casein caused the baked cracker to form a hard outer shell resulting in volume expansion from the trapped steam. Addition of 15 g of the proteins gluten or casein to recipe 1 decreased the formation of acrylamide, but had little effect on its elimination (**Table 1**). Addition of only 1 g of gluten caused a decrease in acrylamide, too. This is possibly due to the reaction of acrylamide with N-terminal amino and cysteinyl sulfhydryl groups (*20*−*22*).

Citric, Hydrochloric, Ascorbic and Ferulic Acids. In agreement with earlier reports (*10*, *23*, *24*), citric acid also decreased the amount of acrylamide produced, as shown in **Table 1**. Larger amounts of citric acid had a greater effect. With little change in elimination, the mechanism likely involves the prevention of formation. The pH of the dough was lowered to 4.6 by addition of 1.25 g of citric acid to recipe 1. To obtain an equivalent decrease in dough pH, 2 mL of 6N HCl was used, and a similar effect on acrylamide level was seen. Thus, many acids could likely decrease the total amount of acrylamide produced in this model cracker, but citric acid is already widely used as a food additive, and might be the preferred choice in real foods.

Ascorbate also increased the elimination of acrylamide and reduced the net amount formed, as shown in **Table 1**. The effect was similar to that of $NaHCO_3$, but slightly less when comparing the same molar concentrations. Ascorbic acid gave similar results, even though the pH of the dough was lower (pH 4.2). The mechanism is undoubtedly complex because of the many thermal decomposition products of ascorbic acid (*25*) and the possibility for free radical reactions (*26*).

Ferulic acid (5 g), or 4-hydroxy-3-methoxycinnamic acid, an antioxidant found in the seeds and leaves of many plants, reduced the net acrylamide formed without affecting the elimination as shown in **Table 1**. This could be due to ferulic acid reacting with acrylamide precursors or intermediates in the production of acrylamide.

Amino Acids and Sugars. Addition of 1 g of asparagine caused an almost 10-fold increase in acrylamide formation, but little change in elimination, as shown in **Table 1**. This supports the hypothesis that asparagine is a source of acrylamide formation. Addition of 1 g of fructose or sucrose had no significant effect on either acrylamide formation or elimination, indicating that asparagine is the limiting precursor in the production of acrylamide in crackers. This is in contrast to results for potatoes, where sugar was found to be limiting. The difference is not surprising considering that wheat has less asparagine than potatoes.

Addition of 1 g of methionine or cysteine decreased the production and increased the elimination of acrylamide (**Table 1**). Most likely, methionine and cysteine were competing with asparagine in reactions that would produce acrylamide. Also, cysteine can react directly with acrylamide, causing its elimination.

Vegetable Oils and Components. The amount of acrylamide produced was affected by the type of vegetable oil used, as shown in **Table 1**. Silicone oil and Niaproof (a surfactant, sodium 7-ethyl-2-methyl-4-undecyl sulfate) were included as inert controls to help separate chemical and physical effects. Compared to recipe 1, all the oils resulted in slightly more acrylamide being produced at 10 and 15 min, while at 20 min the acrylamide was the same or slightly reduced. This suggests the possibility that the cracker interior is heated faster when oils are present. Several oil components (free fatty acids and vitamins, see **Table 1**) were individually tested in an attempt to ascertain the significance of each in contributing to the effect of the oil, but no definitive conclusions could be reached from the preliminary data as they all seemed to have a small effect similar to the oils.

The effect of recipe 2 is shown in **Figure 3**. This recipe included substances that decreased acrylamide when added individually. When added together, much less acrylamide was produced and elimination was increased.

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This work should not be taken as having an impact on FDA policy or regulations.

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