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Investigations of Factors That Influence the Acrylamide Content of Heated Foodstuffs

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The acrylamide content of heated foodstuffs should be considered to be the net result of complex reactions leading to the formation and elimination/degradation of this compound. The present study, involving primarily homogenized potato heated in an oven, was designed to characterize parameters that influence these reactions, including the heating temperature, duration of heating, pH, and concentrations of various components. Higher temperature (200 °C) combined with prolonged heating times produced reduced levels of acrylamide, due to elimination/degradation processes. At certain concentrations the presence of asparagine or monosaccharides (in particular, fructose and also glucose and glyceraldehyde) was found to increase the net content of acrylamide. Addition of other free amino acids or a protein-rich food component strongly reduced the acrylamide content, probably by promoting competing reactions and/or covalently binding acrylamide formed. The dependence on pH of the acrylamide content exhibited a maximum around pH 8; in particular, lower pH was shown to enhance elimination and decelerate formation of acrylamide. In contrast, the effects of additions of antioxidants or peroxides on acrylamide content were small or nonexistent.

KEYWORDS: Acrylamide; cooking; heating; food; potato; Maillard reaction

INTRODUCTION

The discovery (*1*, *2*) that cooking various foods at elevated temperatures results in the formation of acrylamide, at levels as high as milligrams per kilogram in the case of carbohydrate-rich foodstuffs such as potatoes, has caused considerable alarm (*3*). In these primary investigations, factors that influence such formation of acrylamide were examined, partially in order to develop strategies designed to prevent or decrease the extent of formation. Of primary concern in connection with such investigations is that the methodology employed is reproducible and the systems examined are relevant to real-life situations. Some of the parameters that influence chemical reactions in foodstuffs, which are partially heterogeneous and more complex than model solutions, are temperature, the length of the period of heating, pH, the concentrations and reactivity of the components present, and the content of water. However, attempts to develop reproducible and relevant experimental systems are counteracted by variations in, for example, the chemical composition of individual foodstuffs, as is the case for potatoes of different varieties grown under different conditions.

It was proposed (*2*) that the formation of acrylamide in connection with cooking involves the Maillard reaction (*4*, *5*). Obviously, the content of acrylamide detected in a foodstuff is the net result of processes leading to the formation and degradation of this compound. Several research groups have examined acrylamide formation in model systems involving reducing sugars and amino acids (*6*−*9*). Asparagine, which together with glutamine is the most abundant free amino acid in potatoes (*10*, *11*), has been suggested to be a likely source of acrylamide, particularly in potato products (*6*−*8*).

The present investigation was designed to elucidate the effects of certain additions, which alter the pH and concentrations of constituents in potatoes, that might influence the net production of acrylamide. In particular, the effects of natural components (such as sugars, amino acids, and ascorbic acid and also lean meat of fish) were evaluated. Furthermore, preliminary elucidation of the possible role played by antioxidants, free radicals, or other reactive oxygen species in the formation of acrylamide was performed.

MATERIALS AND METHODS

Chemicals. L-Alanine (≥98.5%), L-ascorbic acid (≥99%), L-ascorbic acid sodium salt (sodium ascorbate) (Sigma grade), ascorbic acid 6-palmitate (ascorbyl palmitate) (Sigma grade), l-glutamate monosodium salt (sodium glutamate) (99%), anhydrous L-asparagine (98%),

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d-glucose 6-phosphate monosodium salt (glucose-6-phosphate) (98%), and L-lysine acetate (>98%) were obtained from Sigma (Sigma-Aldrich). 4-Aminobutyric acid (97%), benzoyl peroxide (97%), L-glutamine (≥99%), and D,L-glyceraldehyde (95%) were purchased from Aldrich (Sigma-Aldrich). Glycine (≥99.7%) was procured from Riedel de Haën (Sigma-Aldrich), and d-glucose (≥99%) and D-fructose (≥99%) were from BDH Laboratories (Poole, U.K.). Hydrogen peroxide 30% (p.a.) was obtained from Merck (Darmstadt, Germany), and the anhydrous trisodium salt of citric acid (trisodium citrate, p.a.) from Fisher Chemicals (Leichs, U.K.). All other chemicals and solvents utilized were of analytical grade.

Foodstuffs. All foodstuffs used here were purchased at local grocery stores. Fresh fillet of cod was used for experiments on the day of purchase. Swedish fresh potatoes, of different varieties and from different suppliers, were stored in the dark at room temperature until use. For each individual experiment, the time of purchase, variety, supplier, batch, etc., were recorded, and control samples (i.e., without additions) were always analyzed in parallel. For experiments involving heating in a temperature-programmed oven, frozen ready-made, commercial potato strips of Swedish origin were used. After heating, all of the samples were immediately frozen at −20 °C and stored at this temperature until analysis. The samples were prepared in Stockholm and subsequently transported to Lidköping in dry ice for analysis of their acrylamide content.

Instrumentation. The instrumentation and equipment employed for the workup of samples and liquid chromatographic−tandem mass-spectrometric (LC-MS/MS) analysis of acrylamide were as described previously (*2*). Cooking of foodstuffs was performed by heating as described earlier (*2*) in a thermostated frying pan (*12*) (the surface temperature of which was regulated by a probe; see ref *2*) or a temperature-programmed oven designed for gas chromatography (Hewlett-Packard model 5790A). A Waring blender (model 38 BL41, New Hartford, CT) was utilized for homogenization. Mincing lean meat of fish and grating of potatoes were carried out manually. Following calibration (at 22 °C according to the manufacturer's instructions), an Orion pH-meter (model 290A) equipped with an Orion Ross electrode (model 8102SC) (Thermo Orion) was used for measurement of pH.

Preparation of Homogenized Potato Samples for Heating in an Oven. Tubers of three different varieties were used in these experiments, namely, cv. Frieslander (batch 1, additions of amino acids and carbohydrates; batch 2, addition of hydrochloric acid), cv. Ukama (additions of antioxidants, peroxides, and trisodium citrate), and cv. Rosor (addition of citric acid). For each individual sample, five or six potatoes together weighing ∼500 g were peeled and thereafter chopped into small pieces. Two hundred grams of these pieces was placed in the mixer, various additions (amino acids, carbohydrates, etc.) were then made, and subsequently another 200 g of chopped potato was added. These added amounts were small in relation to the total amount of potato (400 g); deviations from the nominal concentration increments due to changes of total sample mass are negligible. These samples were homogenized for 3 min at speed I (lowest) and then for 3 min at speed II (maximal), resulting in a temperature increase from 22 °C to, typically, 40 °C.

The upper layer of this homogenate, including the foam, was used for pH measurement, whereas the remainder was poured gently into ice-cube trays. The shape and surface of the cubes were made as uniform as possible by gentle adjustment with a spatula. The 16 cubes thus obtained, each weighing ∼15 g, were then frozen at −20 °C. Each sample consisted of three such cubes, which were pooled and, before analysis, homogenized (see below). Soon after being removed from the freezer and without adjustment to room temperature, these samples were heated in a temperature-programmed oven as described below.

Preparation of Samples Containing Mixtures of Fish and Potato. Cod fillet was minced and mixed with peeled and grated potato in different relative proportions (0, 10, 20, 30, 40, 50, or 60 g of fish per patty) to obtain patties weighing a total of 60 g. Directly after preparation seven such samples were heated in a frying pan, and three additional samples were heated in a temperature-programmed oven directly after preparation as described below.

Heating of Potato Samples in an Oven. A temperature-programmed oven designed for gas chromatography was utilized for heating samples consisting of homogenized frozen potato with various ingredients added or of mixtures of fish and potato. For this purpose, the three frozen cubes (trapezoids with a base measuring 25 × 42 mm, a 14 × 30 mm top and a height of 27 mm) of homogenized potato were positioned carefully in a triangle on top of a glass plate (diameter = 92 mm) covered with aluminum foil and with low edges (height = 15 mm). Such careful positioning was found to be important for obtaining reproducible results. Unless otherwise indicated, the temperature program employed was 70 °C for 0.1 min, followed by an increase to 180 °C at a rate of 40 °C/min and, finally, maintenance at 180 °C for 25 min.

Heating of Potato Strips in an Oven. For determination of the time course of acrylamide formation in connection with heating, several frozen, preheated, commercial potato strips of similar size were combined to obtain 30 g samples (cf. ref *2*). The initially frozen samples were carefully positioned and heated in a glass bowl (in the same oven as above) at 70 °C for 0.01 min, followed by an increase to 200 °C at a rate of 40 °C /min, at which temperature the samples were maintained for 1 s or 4, 8, 12, 16, 20, 24, or 60 min. The oven was subsequently cooled to below 70 °C and the samples were weighed and immediately frozen for storage until analysis.

Mass Spectrometric Analysis of Acrylamide. The samples were analyzed as described previously (2): First, the samples were thawed and homogenized, and then water and ($^{13}C_3$)acrylamide (as internal standard) were added; after centrifugation, the supernatant obtained was filtered through a column and the resulting filtrate passed through a syringe filter prior to analysis by LC-MS/MS, with electrospray positive ionization.

In connection with every analytical run, a blank sample consisting of water subjected to the entire workup procedure was also analyzed and the level of detection considered being 3 times the blank value. Reproducibility throughout each analytical series was assured by repeated and alternate analysis of control samples of crisp bread containing 1700 or 85 μg of acrylamide/kg (stored as homogenized samples at −20 °C). If the resolving capacity of the LC column deteriorates, flushing with acetone (0.7 mL/min for 3 h) can be used for regeneration.

This analytical procedure is characterized by a high degree of sensitivity (level of detection ≈ 10 μg/kg), relatively high recovery of the analyte compared to the internal standard [∼100 ± 7.5 (SD)%] for most food samples, and good reproducibility (CV ≈ 5%) (*2*, *13*).

RESULTS AND DISCUSSION

Experimental Reproducibility. The analytical procedure employed, as well as the other experimental conditions and material investigated, must demonstrate acceptable reproducibility. The LC-MS/MS procedure used here is associated with a high degree of reproducibility and stability (see Materials and Methods), as demonstrated by several thousands of analyses performed in our laboratory. In the present experiments, potato samples were the primary material analyzed. The use of an internal standard is essential for obtaining reproducible values, because the recovery varies with different matrices (e.g., due to possible suppression of the formation of certain ions in connection with the LC-MS/MS analysis).

A major problem associated with quantitation of small differences in the acrylamide contents of different samples of heated potato products is the variation in the chemical compositions of potatoes, not only between different varieties but also between individual tubers from the same batch. Other sources of variation in the chemical composition of potatoes (as well as of other vegetables) include their origin, the time period that has elapsed after harvest, and the conditions used for storage (*11*, *14*−*16*). For these reasons, control samples were routinely

Table 1. Effects of Addition of Amino Acids and Sugars on the Net Content[a] of Acrylamide in Samples of Potatoes[a] (Cv. Frieslander, Batch 1) Heated in an Oven at 180 °C for 25 min

addition	amount added (mmol/kg)	measured pH	acrylamide content[b] (μg/kg)	change in acrylamide content (%)
none (control I)		5.82	427	
none (control II)		5.74	542	
none (control III)		5.82	497	
mean (± SD) of controls I–III			488 (± 58.0)	
glutamine	35	5.79	115	–76
	140	5.72	37	–92
glycine	35	5.78	146	–70
	140	5.79	44	–91
4-aminobutyric acid	35	5.81	262	–46
	140	5.86	67	–86
lysine (acetate salt)	35	5.83	276	–43
	140	5.99	59	–88
sodium glutamate	35	5.98	463	–5.3
	140	6.16	226	–54
alanine	35	5.73	422	–14
	140	5.76	243	–50
asparagine	35	5.71	1550	+220
	140	5.61	1885	+290
fructose	35	5.77	2720	+460
	140	5.81	2060	+320
glucose	35	5.76	1280	+160
	140	5.72	691	+41
glucose-6-phosphate	35	5.56	1180	+140
glyceraldehyde	15	5.77	765	+56
	45	5.72	1080	+120

[a] Homogenized potato slurry (single samples). [b] Corrected for weight loss, which was 66.6 ± 1.6% (mean ± SD).

analyzed here; furthermore, to compensate for variations between individual tubers, several different tubers were pooled for homogenization.

Heating experiments must be standardized with regard to both the dimensions and the consistency of the samples to be heated and the temperature and duration of heating. For example, in the heating experiments carried out in this investigation, a standardized positioning of the samples in the oven was found to be important for reproducibility.

In connection with laboratory experiments it is useful to express acrylamide content per kilogram of original sample weight, wherever applicable. Here, the amount of acrylamide determined has been expressed as micrograms per unit weight prior to heating, thus compensating for weight loss (mainly due to evaporation of water) during heating. Because potatoes contain ~80% water, this weight loss was often 50−70% of the fresh weight (see also the next section). Therefore, as consumed, these products contain 2−3 times as much acrylamide per kilogram as indicated by the values presented here. Exceptional loss of weight was considered here to be an indication that the sample in question was an outlier and should thus be disregarded. With respect to control of pH and water loss, heating homogenized samples in an oven is more convenient than heating in a frying pan.

For analysis of single samples with various additions at specified concentrations (**Tables 1** and **4**), the relative standard deviation (coefficient of variation, CV) was assumed to be approximatively the same as for control samples without additions. The CVs were found to be the same (11.9 and 10.1%, respectively) for the series in **Tables 1** and **4**, using different potato cultivars. These CV values were used to estimate the measurement errors of the contrasts between single measurements and controls from the relationship $[(CV^2/n) + CV^2]^{1/2}$ (n = number of controls). The error was estimated to be, in **Table 1**, with $n = 3$, 13.7%, and in **Table 4**, with $n = 4$, 11.3%, with a pooled value 12.5%. Thus, with these varieties observed contrasts greater than ~25% are not likely to be due to measurement error.

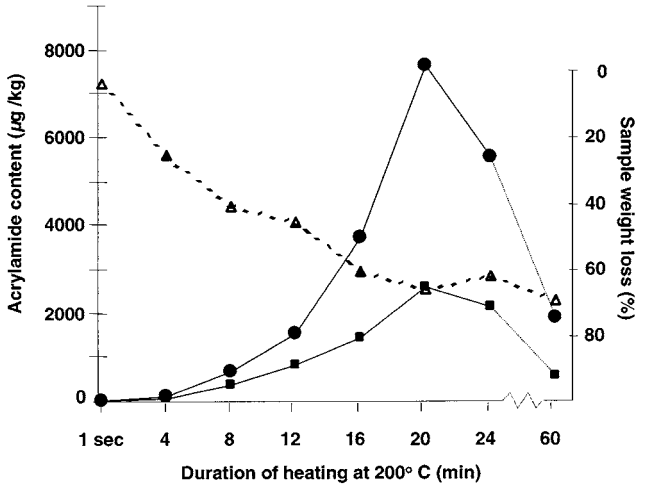

Figure 1. Formation of acrylamide (expressed as micrograms per kilogram) in potato strips heated for different times in an oven at 200 °C. Values both with (squares) and without (circles) compensation for weight loss are presented (indicated by the dotted line with triangles).

Conclusions of this kind with regard to causality are furthermore supported by observations of gradients, for example, in **Table 1**, with mostly three concentrations (background, additions of 35 mM, and additions of 140 mM). Additional support of such conclusions is also obtained by analysis of pooled data for samples with addition of chemicals with related action mechanisms, for example, amino acids, carbohydrates, antioxidants, and pH modulators.

Temperature and Duration of Heating. The influence of temperature on the formation of acrylamide has been repeatedly demonstrated (*2*, *6*−*8*). **Figure 1** illustrates how the acrylamide content of potato strips first increased exponentially with time at a constant oven temperature (200 °C). Then, after prolonged heating, a decrease in the acrylamide content was observed, evidently because degradation of acrylamide (which also occurs in simple model systems; *6*, *8*) becomes predominant (**Figure 1**). **Figure 2** depicts the influence on the acrylamide content, with and without correction for weight loss, in heating French fries in an oven for 15 min at different temperatures of up to 220 °C (data from ref *2*). This dependence of the formation of acrylamide in carbohydrate-rich food on temperature is similar to that observed earlier in protein-rich food, although much lower maximal concentrations are attained in the latter case (*2*).

Effects of Addition of Protein-Rich Components. The influence of addition of lean meat of fish to potato samples was determined in order to examine the possibility that the relatively low levels of acrylamide detected in heated beef or fish products (*2*) might reflect competing or degrading reactions involving proteins. For this purpose, grated potato was mixed with different relative amounts (0−100%) of cod meat and the patties thus formed heated in a frying pan or oven (**Figure 3**). These experiments revealed that the decrease in acrylamide content (by 70% at equal amounts of potato and fish) was considerably greater than would be expected in the case of a purely additive effect (**Figure 3**). This may reflect a protective action of protein, for example, by elimination of acrylamide

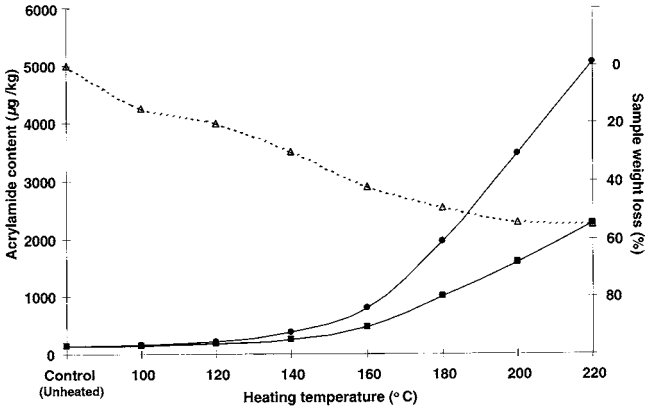

Figure 2. Formation of acrylamide (expressed as micrograms per kilogram) in French fries heated in an oven at different temperatures for 15 min. The temperature program is described in and the data are taken from ref *2*. Values both with (squares) and without (circles) compensation for weight loss (indicated by the dotted line with triangles) are presented.

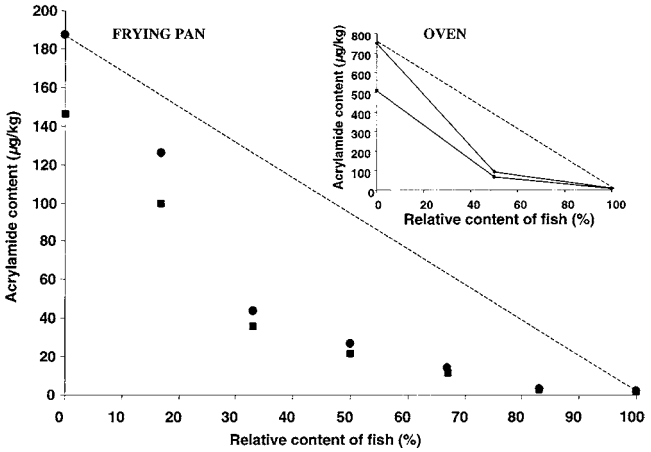

Figure 3. Formation of acrylamide (expressed as micrograms per kilogram) in mixtures of potato and fish upon heating in a frying pan (main figure) or an oven (inset). For details concerning the heating procedure, see Materials and Methods. Values both with (squares) and without (circles) compensation for weight loss are presented. The dashed lines indicate the values expected if protein does not affect acrylamide formation in potato, that is, for a purely additive effect.

formed, perhaps via reaction with nucleophilic groups (−SH, $-NH_2$) on amino acid side chains.

Effects of Adding Amino Acids. In experiments in which free amino acids were added to homogenized potato (cv. Frieslander, batch 1) heated in the oven, the net content of acrylamide was reduced by all of the amino acids tested (glycine, alanine, lysine, glutamine, and glutamic acid) with the exception of asparagine. Additions of 35 mM of these former amino acids resulted in an average decrease in acrylamide levels to approximately half of that observed in the absence of such addition [−42% ± 29% (mean ± SD, $n = 5$)], glutamine and glycine showing the largest reduction (∼−70%). A further lowering occurred with 140 mM [−75% ± 19% (mean ± SD, $n = 5$) (**Table 1**)]. In contrast, 35 mM asparagine strongly enhanced the net acrylamide content and caused an additional small increase at a concentration at 140 mM. The endogenous level of free asparagine in this potato sample (cv. Frieslander, batch 1) was found to be 17.0 mM [as measured (*17*) at AnalyCen AB on frozen potato homogenate and calculated on a wet weight basis], in good agreement with reported values of 15−20 mM free asparagine in potatoes (*10*, *11*). Thus, the amounts of

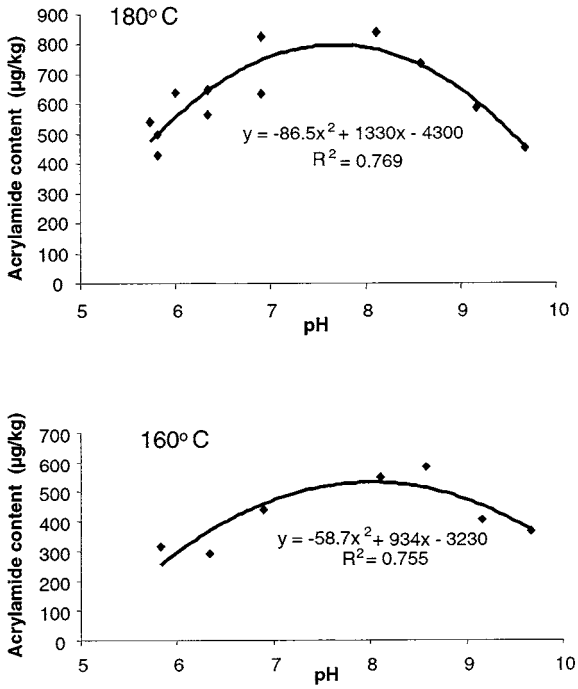

Figure 4. Effects of pH on the content of acrylamide (expressed as micrograms per kilogram with compensation for weight loss) in homogenized potato heated in an oven. The pH was adjusted by addition of NaOH, and heating was carried out at 180 or 160 °C for 25 min.

asparagine added here increased the level of this amino acid by factors of 3 and 9.

Effects of Addition of Carbohydrates. Addition of 35 mM glucose to this same system (i.e., Frieslander potatoes, batch 1, heated in the oven) elevated the net acrylamide content (by 160%), in agreement with findings on model systems (*6*−*9*). However, this enhancement was considerably lower with 140 mM glucose (+41%, **Table 1**). Addition of fructose at 35 mM caused an even higher elevation (+460%), which was again attenuated at the higher concentration. The endogenous contents of free glucose and fructose in this potato sample were found to be 21 and 2.8 mM, respectively, as measured (*18*) at AnalyCen AB and calculated on a wet weight basis. Thus, the additions made here corresponded to increases in the glucose level by 3- and 8-fold and in the fructose level by 14- and 50-fold.

Addition of glyceraldehyde at concentrations of 15 and 45 mM also gave rise to a moderate increase (∼60%) in acrylamide content (**Table 1**). Furthermore, 35 mM glucose 6-phosphate enhanced this content to about the same extent as 35 mM glucose. The attenuation of this effect of glucose at higher concentrations (**Table 1**) resembles the influence of glucose (and of lactose as well) on the formation of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and related food mutagens in heated beef and model systems (*19*, *20*), perhaps reflecting the involvement of similar mechanisms.

pH Effects. Model investigations on the Maillard reaction have revealed that pH is an important determinant of the rate and extent of formation of reaction products, as are temperature, reaction time, water content, and the concentrations and types of reactants present (*4*, *5*). In connection with this reaction the breakdown pathway of Amadori and formation of Heyns rearrangement products have been shown to be pH-dependent (*4*, *5*).

For this reason, the effects of variations of pH on the acrylamide content of homogenized potato after heating were

Table 2. Influence on pH on the Content[a] of Acrylamide in Samples of Potatoes[b] (Cv. Frieslander, Batch 2) Heated in an Oven at 180 °C for 25 min, with and without the Prior Addition of Acrylamide (1000 μg/kg)

measured pH	acrylamide content (μg/kg)	acrylamide content following prior addition of this compound (μg/kg)	loss of added acrylamide (%)
3.0[c]	229	759	47
4.8[c]	356	1050	31
5.7[d]	600	1380	22

[a] Corrected for weight loss. [b] Homogenized potato slurry (single samples). [c] pH adjusted with HCl. [d] No adjustment of pH.

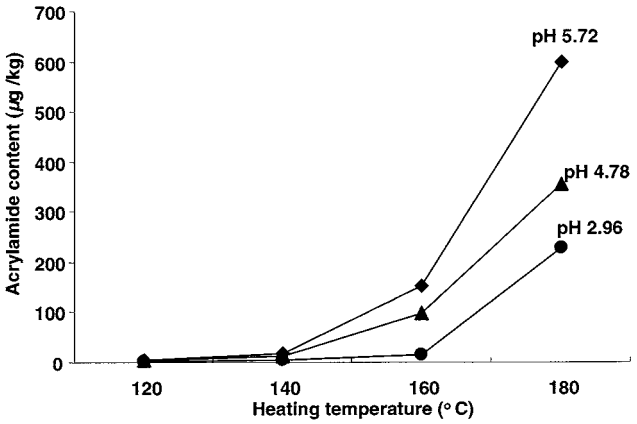

Figure 5. Effect of pH on the content of acrylamide (expressed as micrograms per kilogram with compensation for weight loss) in homogenized potatoes heated in an oven at different temperatures. The pH of the original sample was 5.72, and acidification was achieved by addition of HCl. Heating was carried out for 25 min.

explored here. Lower pH values were attained by the addition of HCl, ascorbic acid, or citric acid, whereas the pH was raised by addition of NaOH. Plotting acrylamide content as a function of pH (**Figure 4**) revealed a maximum value at pH ∼8. The recovery of acrylamide added to these samples prior to heating was reduced at lower pH values (**Table 2** and **Figure 5**), indicating more rapid degradation of this compound in acidified matrices. This effect is probably accompanied by a reduction in the rate of formation. It should be noted that the pH of these samples was measured at ambient temperature prior to heating.

The results of these experiments emphasize the importance of controlling sample pH, which can vary between individual tubers and batches of potato, when such investigations are performed. This is especially important when additions that may modify the pH are made. For this reason, the additives studied here (e.g., amino acids) were, insofar as possible, used in their neutral form.

The extent of protonation of an amino acid at any given pH is determined by the pK_a value of this group, where most such pK_a values for naturally occurring amino acids range between 9.3 and 9.8 (at 25 °C). At a pH of ∼8, the α-amino group of asparagine, which exhibits an exceptionally low pK_a value (≈8.9 at 25 °C), is protonated to a lower degree than is the case for the other amino acids studied here. For this reason, at this pH, asparagine is more prone to react with electrophiles, for example, aldehyde groups in carbohydrates, resulting in Schiff base formation. This reaction is assumed to be the initial step in the formation of acrylamide, in agreement with the comparatively high levels of acrylamide obtained upon addition of asparagine or carbohydrates (**Table 1**), findings that confirm the results of model experiments involving heating of reducing sugars in the presence of asparagine (*6*, *7*).

Table 3. Effects of the Addition of Citric Acid on the Acrylamide Content[a] in Samples of Potatoes[b] (Cv. Rosor) Heated in an Oven at 180 °C for 25 min

sample addition	amount added (mmol/kg)	measured pH	acrylamide content (μg/kg)
none (controls)		5.75	275
citric acid	6.5 (0.125)[c]	5.61	225
	26 (0.500)	5.24	180
	104 (2.00)	4.54	149

[a] Corrected for weight loss. [b] Homogenized potato slurry (single samples). [c] Percent of wet weight is given in parentheses.

Table 4. Effects of Sodium Ascorbate, Ascorbyl Palmitate, Bensoyl Peroxide, and Hydrogen Peroxide on the Acrylamide Content[a] of Homogenized Potatoes[b] (Cv. Ukama) Heated in an Oven at 180 °C for 25 min

addition	amount added (mmol/kg)	measured pH	wt loss (%)	acrylamide content (μg/kg)	change in acrylamide content (%)
none (control I)		5.70	68.8	407	
none (control II)		5.78	70.3	477	
none (control III)		5.92	67.3	396	
none (control IV)		5.89	67.7	476	
mean ± SD for controls I–IV				439 (±43.5)	(±10)
sodium ascorbate	1.51	5.76	67.3	494	+12
	4.54	5.81	64.6	429	−2.3
	13.6	6.05	66.4	379	−14
	40.9	5.88	65.6	422	−3.9
	123	6.01	61.5	239	−46
ascorbyl palmitate	1.51	5.86	65.1	569	+30
	4.54	5.8	65.5	499	+14
	13.6	5.57	65.1	491	+12
benzoyl peroxide	1.03	5.73	68.4	640	+46
	4.13	5.73	67.8	501	+14
hydrogen peroxide	6.0	5.77	62.3	453	+3.2
	18	5.6	67.3	510	+16
	54	5.74	64.8	452	+3.0
	162	5.66	68.3	470	+7.1
trisodium citrate	4.54	5.96	65.1	442	+0.7
	13.6	6.06	64.1	537	+22
	40.9	6.29	62.2	283	−36
	123	6.53	60.4	208	−53

[a] Corrected for weight loss. [b] Homogenized potato slurry (single samples).

Effects of Acidic Food Additives. To study the effects of additives that modify the pH of foodstuffs on acrylamide content, citric acid was added to homogenized potato prior to heating in the oven (as described above) (**Table 3**). The effects of such addition resembled those observed upon acidification with HCl.

Influence of Antioxidants, Oxidants, and Radical Initiators. Ascorbic acid has been shown to behave as a reducing sugar in the Maillard reaction, where its degradation products react with amino acids and peptides (*21*, *22*) and inhibit browning (*23*, *24*).

To examine the effects of antioxidants on the acrylamide content of potato samples heated in the oven, two antioxidants that are accepted food additives were admixed into these samples. The possible influence of the polarity could be evaluated by comparison effects of the lipophilic ascorbyl palmitate and the hydrophilic sodium ascorbate. At the lowest concentration tested (1.51 mM), both of these antioxidants caused a slight increase in the acrylamide content, exceeding

Table 5. Parameters Examined with Respect to Their Influence on the Formation/Elimination of Acrylamide during Cooking

parameter	relative effect on acrylamide content	interpretation
increased temperature	+	pronounced enhancement of net formation
longer time at high temperature	+	prolonged time at high temperatures strongly enhances the rate of acrylamide formation, but at longer times there is a decrease in the net content of acrylamide; the rates of competing reactions involved in formation and elimination reactions are assumed to vary during the process of heating
pH	+/–	optimal formation at around pH 8; at higher and, especially lower pH values, the rate of formation and/or the rate of elimination/dedgradation increased
addition of monosaccharides	+	strong increment; fructose is the most effective precursor, with glucose and glucose 6-phosphate being less effective; the effects are decreased with higher concentrations
addition of asparagine	+	pronounced increment; decreased effect at higher concentrations; excellent precursor
addition of amino acids other than asparagine	–	pronounced reduction, probably due to competitive consumption of precursors and/ /or increased elimination/degradation
addition of antioxidants or radical initiators	(+)?	involvement of radicals or peroxidation in the formation of acrylamide may be of some, but only minor, importance; low levels of antioxidants cause a small increase (via protection of the acrylamide formed?); a small increase was also attained with low amounts of the radical initiator benzoyl peroxide; addition of H_2O_2 gave no significant effect
addition of components that bind water	–	reduction in acrylamide levels, due to reduced pyrolysis of the sample when large amounts of water-binding components were present
addition of lean meat of fish	–	strong reduction of the acrylamide content of heated potato samples (probably due to elimination of acrylamide by reaction with or adsorption to protein)

the estimated error (**Table 4**). At higher concentrations added, the changes in the acrylamide content were smaller except for the highest concentration, tested with ascorbate only. This concentration of ascorbate (123 mM, 24.4 g/kg) provoked a strong reduction of the acrylamide content (−46%) that might be considered significant. This phenomenon is unlikely to involve the antioxidative effect of ascorbate, because a similar decrease by −53% was also observed with 123 mM trisodium citrate, which is not an antioxidant (**Table 4**). It appears more likely that addition of large amounts of this kind of salt promotes binding of water (reflected as decreased weight loss; **Table 4**), thus leading to partial inhibition of the pyrolysis reaction (observed visually here as reduced browning).

In another test, somewhat different from that described above, ascorbic acid (0.30, 1.7, or 8.3 wt %) was added to the potato sample (30 g), which was then homogenized and immediately heated in a microwave oven, for 3 min at 750 W as described earlier (*2*). In this case, the acrylamide level was reduced from 3100 μg/kg with 0.30% ascorbic acid to 420 μg/kg with 1.7% to below the limit of detection (50 μg/kg in this study) upon addition of 8.3%. This dramatic decrease cannot be accounted for simply by changes of pH, which were found to be 5.19, 4.10, and 3.35, respectively. A more reasonable explanation could be the combined effects of the lowering of pH and the binding of water (compare the effects of trisodium citrate and sodium ascorbate described above). In connection with microwave heating the presence of even relatively small amounts of water exerts a pronounced effect on preventing the pyrolysis of samples. In an earlier study involving the same system as employed above (*2*), an increase in the duration of microwave heating from 100 to 150 s resulted in an elevation of the acrylamide level from 47 μg/kg (61% weight loss) to 4400 μg/kg (77% weight loss).

The effects of hydrogen peroxide (which is both an oxidant and a radical initiator) and of benzoyl peroxide (radical initiator) were also examined. A probably real increase (+46%) in acrylamide content was obtained in the presence of 1 mmol benzoyl peroxide ($n = 1$), whereas the effect of the addition of 4 mmol/kg was in the range of the estimated error. Furthermore, addition of increasing concentrations of hydrogen peroxide did not alter the content of acrylamide observed following heating (**Table 4**). These findings indicate that peroxidation or formation of free radicals, in the absence of frying oils, plays no major role in the acrylamide formation associated with heating.

Conclusions. On the basis of the present investigation, **Table 5** summarizes observed influences of various factors on the acrylamide content of heated foodstuffs. In general, our present studies involving foodstuffs lend support to the conclusions drawn from simple model experiments, that is, that acrylamide can be formed by reaction of the amino acid asparagine with glucose or fructose. In the experiments with potato reported here, fructose was seen to be a more efficient precursor in this regard than glucose or glucose-6-phosphate. The observed influence of added amino acids on the acrylamide content illustrates the complexity of the underlying processes in heated foodstuffs, in terms of the reactions that are rate limiting with respect to the formation of acrylamide, and the presence of inhibitors and/or competing reactions. In addition to these reactions, other nucleophilic components might also eliminate acrylamide. The reactivity of acrylamide has recently been discussed by Friedman (*25*).

Furthermore, our present findings lead us to propose that additives that have the capacity to bind water reduce the net formation of acrylamide, because water inhibits pyrolysis reactions. In this context, studies designed to elucidate the influence of the initial water content of samples on the formation of acrylamide during heating should be performed.

Acrylamide is classified as a probable human carcinogen (*26*). An improved understanding of the reactions leading to acrylamide formation and degradation/elimination during heating will facilitate development of methods for decreasing the acrylamide content of cooked food.

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