Changes in Free Amino Acids and Sugars in Potatoes Due to Sulfate Fertilization and the Effect on Acrylamide Formation

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To examine how sulfur deprivation may affect acrylamide formation in cooked potatoes, three varieties of potato were grown under conditions of either severe sulfur deprivation or an adequate supply of sulfur. In all three varieties sulfur deprivation led to a decrease in acrylamide formation, even though the levels of sugars, which are acrylamide precursors, were higher in tubers of the sulfur-deprived plants. In one variety the concentration of free asparagine, the other precursor for acrylamide, was also higher. There was a very close correlation between the concentration of asparagine in the tubers expressed as a proportion of the total free amino acid pool and the formation of acrylamide upon cooking, whereas sugars were poorly correlated with acrylamide. In potatoes, where concentrations of sugars are usually limiting, competition between asparagine and other amino acids participating in the Maillard reaction may be a key determinant of the amount of acrylamide that is formed during processing.

KEYWORDS: Acrylamide; asparagine; potato; sugars; agronomy; sulfate fertilizer

INTRODUCTION

The formation of significant levels of the suspected carcinogen acrylamide in heated foods high in carbohydrate, including those derived from potato, wheat, maize, and other cereals, as well as coffee, has been widely reported. Acrylamide is produced by a reaction between free asparagine and intermediates of the Maillard reaction, the major precursors therefore being asparagine and sugars. There has been a lot of interest in how food-processing conditions may be altered to reduce acrylamide levels in food products (*1, 2*). However, there is a limit to what can be achieved by this approach, in part because the Maillard reaction is also responsible for the generation of desirable flavors and colors in heated food.

Relatively little attention has been paid to how agronomic practice might be altered to reduce the levels of acrylamide precursors in crop plants, but several studies have shown that such an approach could make significant improvements. For example, Muttucumaru and co-workers showed that the concentration of free amino acids was much higher in flour from wheat grown under sulfur-deficient conditions than in flour from wheat grown with sufficient amounts of sulfur (*3*), with up to 30 times more free asparagine. On heating at 160 °C for 20 min, levels of acrylamide in the flour from the sulfur-deprived

wheat were >5 times higher than that in the control. Nitrogen application to wheat has the opposite effect to that of sulfur: Claus and co-workers showed that increasing nitrogen application led to increased levels of asparagine, resulting in increased acrylamide in heated flour and bread (*4*). The data of Muttucumaru and co-workers showed a clear relationship between wheat grain asparagine levels and acrylamide formation (*3, 5*), indicative of the high levels of sugars relative to free amino acids in cereals.

Two studies have reported somewhat contradictory results on the effect of soil nitrogen on potatoes. De Wilde and co-workers showed that the levels of tuber sugars rose in nitrogen-deprived potatoes by up to 100% compared with adequately fertilized potatoes, resulting in an increase in acrylamide in French fries of up to 65% (*6*). In contrast, Amrein and co-workers found no correlation between the amount of nitrogen fertilization and acrylamide formation in 57 samples of cooked, grated potato, from 10 different cultivars (*7*). In potato tubers, the ratio of sugars to free amino acids is much lower than that in cereal grain, and it has been reported that sugars, not asparagine, limit acrylamide formation in potato-based products (*7, 8*).

Here we report on the effect of sulfate deprivation on the free amino acid and sugar contents of three different potato varieties. As well as showing clear differences between varieties, the results indicate a more complex relationship between the amounts of asparagine and sugars in potatoes and the formation of acrylamide than has been proposed previously.

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MATERIALS AND METHODS

Reagents and chemicals were purchased from normal laboratory suppliers and were of analytical grade. [$^{13}C_3$]Acrylamide (1 mg/mL in methanol) was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA).

Growing of Potato Plants. Three varieties of potato, King Edward, Prairie, and Maris Piper, were grown in a glasshouse in pots containing vermiculite. Day temperature was maintained at 18 °C and night temperature at 16 °C; supplementary lighting was used to provide the plants with a 16 h day, and feeding started on shoot emergence. Ten plants were grown of each variety; five were watered with medium containing sufficient amounts of potassium, phosphate, calcium, magnesium, sodium, iron, nitrate, and sulfate ions (1 mM $MgSO_4$), and five were watered with the same medium lacking the sulfate. Tubers were harvested at maturity, with the haulms intact, approximately 12 weeks after planting. Within 24 h of harvesting, the tubers were peeled, sliced, and placed in aluminum trays, frozen in a blast freezer for 30 min, and freeze-dried for 3 days. The dried samples were powdered using a food processor, vacuum sealed separately in pouches, and stored at −18 °C until analysis.

Determination of Free Amino Acids. A tuber flour sample (0.500 ± 0.005 g) was weighed into a 100 mL screw-top bottle. Hydrochloric acid (50 mL, 0.01 M) was added to the vial, and the sample was stirred for 15 min at room temperature. An aliquot of supernatant (2 mL) was centrifuged at 7200g for 15 min. The amino acids in 100 μL of the centrifuged supernatant were then derivatized using the EZ-Faast amino acid derivatization technique for GC-MS (Phenomenex, Torrance, CA) (*9*). Arginine could not be measured using this technique.

GC-MS analysis of the derivatized sample was carried out using an Agilent 5975 system (Agilent, Palo Alto, CA) in electron impact mode. An aliquot of the derivatized amino acid solution (1 μL) was injected at 280 °C in split mode (40:1) onto a Zebron ZB-AAA capillary column (10 m × 0.25 mm; 0.25 μm film thickness). The oven temperature was held at 110 °C for 1 min and then increased at 30 °C/min to 310 °C. The transfer line and ion source were maintained at 320 and 230 °C, respectively; carrier gas flow rate was kept constant throughout the run at 1.5 mL/min. Three analyses were performed for each sample.

Analysis of Sugars by Ion Chromatography. Each tuber flour sample (0.200 ± 0.005 g) was weighed into a 14 mL screw-top bottle. Aqueous methanol (50%) containing 100 mg/L trehalose as an internal standard was added to the bottle, and the sample was stirred for 15 min at room temperature. After a further 15 min, 1.5 mL of supernatant was removed from the bottle and centrifuged at 7200g for 15 min. Aliquots (500 μL) of the centrifuged supernatant were diluted 10-fold in water; 2 mL of the diluted extract was then filtered through a 0.2 μm syringe filter.

The extracts were analyzed using a Dionex ion chromatography system with a 250 × 4 mm Carbopac PA1 column (Dionex Corp., Sunnyvale, CA), operated using Chromeleon software. The ion chromatography system consisted of an AS50 autosampler, an LC25 column oven, GS50 pumps, and an ED50 pulsed amperometric detector, running in internal amperometric mode. Injection volume was 25 μL. A gradient program was set up using 200 mM NaOH (solvent A) and water at a flow rate of 1 mL/min: 50% solvent A, held for 10 min and then increased to 100% at 40 min. The column was then washed for 8 min with 500 mM sodium acetate in 125 mM NaOH and re-equilibrated with 50% solvent A for 7 min. The waveform of the pulsed amperometric detector was as follows: 400 ms at 0.1 V, 20 ms at −2.0 V, 10 ms at 0.6 V, and 60 ms at −0.15 V. Standards of glucose, fructose and sucrose were used for quantification. Each sample was extracted and analyzed in triplicate.

Production and Analysis of Acrylamide. Tuber flour samples (0.5 g) in unsealed glass ampules (1 mL capacity) were heated for 20 min at 160 °C. Copper wire was tied around the necks of the filled ampules and the ampules were heated by being suspended by the wire from the ceiling of a GC oven. Acrylamide was extracted from these samples with 25% aqueous methanol and converted to the dibromo derivative prior to analysis by GC-MS, using the method of Castle et al. (*10*), with the modifications described by Elmore et al. (*9*). Labeled [$^{13}C_3$]acrylamide was used as the internal standard.

The brominated extracts (2 μL) were injected onto the Agilent 5975 GC-MS system in pulsed splitless mode at 250 °C, the splitter opening after 0.5 min. The helium carrier gas pressure was 21 psi in pulsed mode, falling to 9.6 psi for the rest of the run. A DB-17 MS capillary column was used (30 m × 0.25 mm i.d., 0.15 μm film thickness; Agilent). The oven temperature was 85 °C for 1 min, rising at 8 °C/min to 200 °C and then at 30 °C/min to 280 °C for 10 min. The transfer line was held at 280 °C and the ion source at 180 °C. The mass spectrometer was operated in electron impact mode with selected ion monitoring. Two ions were used to monitor brominated [$^{13}C_3$]acrylamide (m/z 153 and 155), and another two ions were used for brominated acrylamide (m/z 150 and 152). The ion m/z 155 was used to quantify brominated [$^{13}C_3$]acrylamide, and the ion m/z 150 was used to quantify brominated acrylamide. Each sample was prepared and analyzed in triplicate.

RESULTS AND DISCUSSION

Three varieties of potato, King Edward, Prairie, and Maris Piper, were grown in pots and watered with medium containing sufficient amounts of the normal nutrients with the exception of sulfate, which was supplied to only half of the plants of each variety. There was a general rise in the total level of free amino acids in the tubers in response to sulfur deprivation (**Table 1**); in the case of Prairie the increase was almost 3-fold. However, there was a clear difference between the varieties with respect to the predominant amino acid that accumulated. Sulfur-deprived plants of the King Edward and Maris Piper varieties accumulated a particularly high level of glutamine in the tubers (in King Edward, for example, the glutamine level rose from 80.6 to 293 mmol/kg of dry weight), whereas the asparagine level fell (in King Edward from 177 to 95.4 mmol/kg of dry weight). In contrast, whereas the tubers of the Prairie variety also accumulated a high level of glutamine in response to sulfur deprivation (93.0 mmol/kg of dry weight, compared with 12.4 mmol/kg of dry weight in tubers of sulfur-fed plants), they also showed a large increase in asparagine (126 mmol/kg of dry weight in tubers of sulfur-deprived plants compared with 62.5 mmol/kg of dry weight in tubers of sulfur-fed plants).

All of the other measured amino acids, apart from aspartic acid and β-alanine, increased significantly in the sulfur-deprived samples. In each variety, leucine, isoleucine, ornithine, phenylalanine, tryptophan, and lysine were all present at levels at least 5 times higher in the sulfur-deprived samples compared with their levels in the sulfur-fed samples.

In all three varieties, sulfur deprivation also caused an increase in the levels of sugars (**Table 2**). Notably, the concentration of fructose more than doubled in Prairie tubers, whereas glucose increased from 1.74 to 7.81 mmol/kg of dry weight in Maris Piper tubers. Sucrose is the predominant sugar in potato tubers, but as a nonreducing sugar it does not react directly with amino acids. However, at frying and baking temperatures sucrose will undergo decomposition to reactive carbonyl compounds. Stadler and co-workers reported approximately equal reactivities of fructose, glucose, and sucrose in model reactions with asparagine at a temperature of 180 °C (*11*), whereas Claeys and co-workers found sucrose to be about 50% as reactive toward asparagine as glucose on a molar basis over temperatures from 140 to 200 °C (*12*). Sucrose must therefore be included in discussions of sugars as acrylamide precursors. The sucrose level hardly changed in King Edward but increased with sulfur deprivation in the other two varieties, and whereas the percentage increase was smaller than for the other sugars measured, it was responsible for most of the overall increase in sugars.

Despite these increases in sugars and, in the case of Prairie, an increase in asparagine, acrylamide formation in tuber flour

Acrylamide in Potatoes in Relation to Amino Acid Composition

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Table 1. Free Amino Acids (Millimoles per Kilogram of Dry Weight) in Tuber Material from Three Varieties of Potato, Grown in Pots on Vermiculite with (S+) or without (S−) Added Magnesium Sulfate[a]

amino acid	King Edward		Prairie		Maris Piper	
	S+	S−	S+	S−	S+	S−
asparagine	177 (9.05)	95.4 (3.33)	62.5 (3.78)	126 (1.59)	91.6 (1.34)	51.8 (0.45)
glutamine	80.6 (5.18)	293 (10.3)	12.4 (1.63)	93.0 (1.43)	27.5 (2.56)	134 (14.2)
tryptophan	0.09 (0.01)	0.89 (0.16)	0.02 (0.01)	0.31 (0.05)	0.10 (0.02)	1.04 (0.04)
alanine	6.33 (0.10)	26.7 (0.34)	1.22 (0.03)	3.10 (0.13)	1.60 (0.04)	7.25 (0.07)
glycine	1.66 (0.11)	6.29 (0.23)	0.40 (0.02)	1.22 (0.04)	0.76 (0.07)	11.9 (0.37)
valine	6.00 (0.06)	20.6 (0.85)	1.91 (0.04)	12.1 (0.15)	4.02 (0.15)	22.6 (0.45)
β-alanine	0.56 (0.17)	0.43 (0.01)	0.14 (0.08)	0.11 (0.20)	0.21 (0.05)	0.66 (0.11)
threonine	2.43 (0.39)	9.25 (0.84)	0.58 (0.07)	1.67 (0.23)	1.06 (0.10)	5.61 (0.52)
serine	11.3 (0.61)	70.1 (3.71)	0.90 (0.01)	3.78 (0.32)	3.43 (0.22)	22.1 (1.14)
proline	24.6 (1.09)	39.8 (1.74)	4.09 (0.43)	13.4 (0.17)	6.48 (0.21)	8.95 (0.55)
aspartic acid	11.3 (0.05)	11.4 (0.50)	8.74 (0.74)	8.74 (0.28)	9.35 (0.21)	7.44 (0.62)
methionine	1.54 (0.11)	4.99 (0.25)	1.14 (0.05)	2.70 (0.11)	2.27 (0.14)	6.03 (0.77)
glutamic acid	32.2 (5.42)	40.0 (1.77)	13.4 (1.47)	20.3 (2.68)	21.4 (0.84)	18.0 (0.88)
phenylalanine	1.44 (0.08)	10.4 (0.44)	0.54 (0.04)	8.49 (0.14)	1.04 (0.06)	10.7 (0.23)
GABA	4.98 (0.26)	6.82 (0.19)	2.86 (0.43)	4.24 (0.82)	5.10 (0.39)	4.77 (0.60)
ornithine	0.09 (0.02)	0.47 (0.03)	0.09 (0.01)	1.04 (0.18)	0.13 (0.01)	2.03 (0.19)
lysine	0.21 (0.02)	2.60 (0.17)	0.20 (0.05)	2.26 (0.12)	0.45 (0.06)	3.80 (0.08)
histidine	0.27 (0.03)	0.73 (0.19)	0.13 (0.02)	0.57 (0.22)	0.15 (0.05)	0.59 (0.09)
tyrosine	0.47 (0.03)	11.2 (0.09)	0.03 (0.02)	0.05 (0.29)	0.10 (0.04)	6.65 (0.25)
leucine	0.37 (0.02)	4.69 (0.13)	0.20 (0.01)	2.48 (0.06)	0.35 (0.01)	4.41 (0.05)
isoleucine	0.79 (0.01)	7.03 (0.23)	0.40 (0.04)	4.29 (0.12)	0.88 (0.08)	8.06 (0.12)
total	364	663	112	310	178	338

[a] Values are means of three replicates with standard deviations in parentheses.

Table 2. Sugars (Millimoles per Kilogram of Dry Weight) in Tuber Material from Three Potato Varieties Grown with or without the Addition of Sulfate[a]

sugar	King Edward		Prairie		Maris Piper	
	S+	S−	S+	S−	S+	S−
glucose	1.62 (0.06)	2.42 (0.48)	1.18 (0.25)	1.45 (0.25)	1.74 (0.42)	7.81 (0.18)
fructose	1.11 (0.16)	2.04 (0.29)	0.509 (0.10)	1.12 (0.25)	1.45 (0.31)	2.10 (0.20)
sucrose	29.6 (0.24)	29.7 (0.64)	28.9 (1.31)	34.5 (2.33)	27.4 (0.40)	37.4 (0.63)
total	32.3	34.1	30.6	37.1	30.6	47.3

[a] Values are means of three replicates with standard deviations in parentheses.

Table 3. Acrylamide (Micrograms per Kilogram, Uncooked Weight) Formed in Potato Flour from Three Potato Varieties Grown with or without the Addition of Sulfate[a]

	King Edward		Prairie		Maris Piper	
	S+	S−	S+	S−	S+	S−
acrylamide	4063 (134)	1417 (84.8)	3351 (273)	2254 (61.3)	3567 (123)	1529 (43.9)

[a] The tuber material was heated at 160 °C for 20 min. Values are means of three replicates with standard deviations in parentheses.

from the sulfur-deprived plants was always lower than in tuber flour from the sulfur-fed plants (**Table 3**). In the King Edward variety, sulfur deprivation reduced acrylamide formation by almost two-thirds, from 4060 to 1420 μg/kg. A possible explanation for this unexpected result became apparent when the levels of acrylamide formed on heating were plotted against the concentration of asparagine expressed as a percentage of the total free amino acid pool (**Figure 1**) and a close correlation was revealed ($R^2 = 0.8354$). In other words, changes in asparagine concentration per se and in sugars were outweighed by the large increase in the size of the total amino acid pool (**Table 1**), resulting, in the main, from the increase in glutamine concentration.

Sugars have been shown to be a limiting factor for acrylamide formation in potato products (*7*, *8*), and whereas sugar levels in the tubers from the sulfur-deprived plants were up to 55% higher than in tubers from the sulfur-fed plants, total free amino acids (excluding arginine) were between 82 and 176% higher.

We propose that because the sugars could react with any of the other amino acids present, the amount of sugar available to react with asparagine was lower in the sulfur-deprived samples and hence acrylamide production was reduced.

These data raise some difficult questions regarding potato husbandry. Sulfur nutrition, once of little concern in the United Kingdom, is becoming an increasing problem. This is probably because of a decrease in the use of sulfur-containing fertilizers, such as ammonium sulfate and superphosphate (a mixture of dihydrogen phosphate and hydrated calcium sulfate), increased crop yields resulting in the removal of minerals from the soil, and reduced atmospheric deposition, due to a switch away from coal as a fuel to low-sulfur or sulfur-free alternatives, such as natural gas (*13*). Sulfur deficiency has also been reported in northern and western Europe, Australia, and New Zealand (*14*, *15*). The data presented here show clearly that, in contrast to wheat (*4*), sulfur feeding of potato leads to an increase in acrylamide formation during processing. Clearly, a difficult

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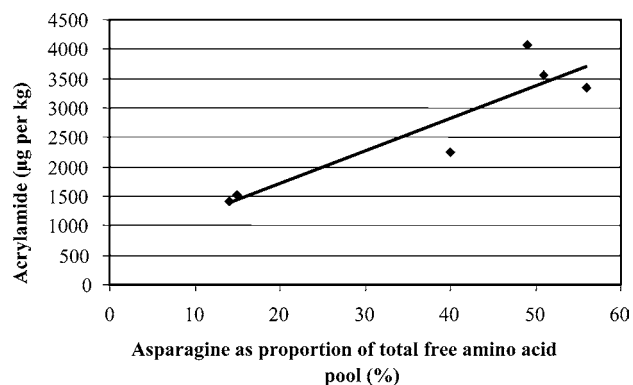
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Figure 1. Relationship between asparagine concentration expressed as a proportion of the total free amino acid pool and acrylamide formation in potato flour, after heating at 160 °C for 20 min ($R^2 = 0.8354$).

balance may have to be struck between keeping soil sulfur levels high enough to maintain the health of the crop while not exacerbating the problem of acrylamide formation.

The data also cast doubt on the wisdom of current advice for plant breeders for addressing the acrylamide problem. In contrast to the data presented in two other studies (*7*, *8*), which suggested that the level of sugars was the key parameter for acrylamide formation in potato, the relationship between sulfur feeding and acrylamide formation in this study did not correlate with a corresponding increase in sugars. Indeed, in all three varieties studied the level of sugars in the tubers fell in response to sulfur feeding. However, whereas the absolute concentration of asparagine rose in the King Edward and Maris Piper varieties yet fell in Prairie, there was a close correlation between the concentration of asparagine as a percentage of the total amino acid pool and acrylamide formation. This suggests that when the sugar level is limiting, as it is in potato tubers, competition between asparagine and other amino acids for participation in the Maillard reaction may be a key determinant of the amount of acrylamide that is formed during processing. This hypothesis is consistent with the effect of adding glycine or glutamine to potato or cereal products before heating, where a reduction of up to 90% in acrylamide formation is possible (*16*).

Changes in the amino acid profile of potato tubers may affect the flavor and/or color of the product. In a model system, for example, the addition of glycine has been shown not only to reduce the formation of acrylamide but also to change the profile of alkylpyrazines that are formed and suppress the formation of Strecker aldehydes, both key flavor components (*17*). Nevertheless, genetic interventions or changes in agronomic practice that induce potatoes to store other amino acids in preference to asparagine clearly have a high likelihood of success in mitigating the acrylamide problem.

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