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Effects of Water Availability on Free Amino Acids, Sugars, and Acrylamide-Forming Potential in Potato

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Ⓢ Supporting Information

ABSTRACT: Irrigation is used frequently in potato cultivation to maximize yield, but water availability may also affect the composition of the crop, with implications for processing properties and food safety. Five varieties of potatoes, including drought-tolerant and -sensitive types, which had been grown with and without irrigation, were analyzed to show the effect of water supply on concentrations of free asparagine, other free amino acids, and sugars and on the acrylamide-forming potential of the tubers. Two varieties were also analyzed under more severe drought stress in a glasshouse. Water availability had profound effects on tuber free amino acid and sugar concentrations, and it was concluded that potato farmers should irrigate only if necessary to maintain the health and yield of the crop, because irrigation may increase the acrylamide-forming potential of potatoes. Even mild drought stress caused significant changes in composition, but these differed from those caused by more extreme drought stress. Free proline concentration, for example, increased in the field-grown potatoes of one variety from 7.02 mmol/kg with irrigation to 104.58 mmol/kg without irrigation, whereas free asparagine concentration was not affected significantly in the field but almost doubled from 132.03 to 242.26 mmol/kg in response to more severe drought stress in the glasshouse. Furthermore, the different genotypes were affected in dissimilar fashion by the same treatment, indicating that there is no single, unifying potato tuber drought stress response.

KEYWORDS: acrylamide, asparagine, drought, free amino acids, potato, sugars, processing contaminants, food safety

■ INTRODUCTION

The ability of crops to tolerate abiotic stresses such as an inadequate supply of water is an important aspect of crop yield resilience and food security and has long been a target for plant breeders. It is now becoming clear, however, that the impact of water availability and other stresses on crop composition is just as important as its effect on yield. The composition of a crop product affects its processing properties and the nutritional value of the food that is produced from it. Crucially, in some cases it also affects food safety and regulatory compliance, with the potential for formation of undesirable processing contaminants being determined by the composition of the raw crop product.[1,2]

The most important processing contaminant for potato (*Solanum tuberosum*) is acrylamide, which forms within the Maillard reaction, a series of nonenzymic reactions between reducing sugars and amino groups during high-temperature cooking (frying, baking, and roasting) and processing.[3−5] It results in the formation of a plethora of products, many of which impart color, aroma, and flavor, but acrylamide forms when asparagine participates in the reaction.[6−8] Free asparagine and reducing sugars can therefore be regarded as the precursors for acrylamide, but it should be noted that, whereas this appears to be the major route for acrylamide formation, others have been proposed, for example, with 3-aminopropionamide as a possible transient intermediate[9,10] or, in cereals, through pyrolysis of gluten.[11]

Acrylamide has been classified as a group 2A, "probably carcinogenic to humans", chemical by the International Agency for Research on Cancer[12] because of the carcinogenicity it has shown in rodent toxicology studies,[13,14] and the latest report on dietary acrylamide from the European Food Safety Authority (EFSA)'s Expert Panel on Contaminants in the Food Chain (CONTAM) described it as potentially increasing the risk of developing cancer for consumers in all age groups.[15] The Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) Joint Expert Committee of Food Additives (JECFA) has also concluded that the presence of acrylamide in the human diet is a concern.[16] In addition to its carcinogenic properties, acrylamide has neurological, reproductive, and developmental effects at high doses, but CONTAM considered these not to be a concern at current levels of dietary exposure.[15]

In Europe, the contribution of potato products for adults (18−64 years) ranges from 18.3% of the total in France to 67.1% in the United Kingdom (UK).[17] Most of this intake comes from French fries, with the rest from chips (UK crisps) and oven-cooked potatoes.[17] These products are among those

for which the European Commission has issued "indicative" levels for the presence of acrylamide.[18]

The European food industry has devised many strategies for reducing acrylamide formation by modifying food processing. These have been compiled in a "*Toolbox*" produced by Food Drink Europe.[19] Analysis of manufacturers' data on acrylamide levels in potato chips in Europe showed a clear, statistically significant downward trend for mean levels of acrylamide from 763 (±91.1) μg/kg (parts per billion) in 2002 to 358 (±2.5) μg/kg in 2011, a decrease of 53% (±13.5), which was taken as evidence of the effectiveness of the "*Toolbox*".[20] However, the effect of seasonality arising from the influence of potato storage on acrylamide levels was evident in the study, and this was consistent with the results of a study that had analyzed samples of commercial potatoes in the United Kingdom from harvest through 9 months of storage,[21] showing the difficulty of processing a variable raw material to give a consistently low acrylamide level in the product. In the United States, the Food and Drug Administration has developed an "action plan" with a number of goals, including identifying means to reduce exposure. A North American perspective on the issue and the response to it has been given by Bethke and Bussan.[22]

Reducing the acrylamide-forming potential of potatoes and making it more consistent would be a great help to the food industry. In the United States, The J. R. Simplot Co. has recently begun to market a low-acrylamide biotech potato variety that has reduced activity of an asparagine synthetase gene (*ASN1*), two genes encoding enzymes of starch breakdown, phosphorylase L (*PhL*), and starch-associated R1 (*R1*), as well as a gene (*PPO5*) encoding polyphenol oxidase, an enzyme involved in bruising.[23,24] There is currently no possibility of such an approach being taken in Europe, but plant and agronomic science still have an important part to play, for example, through the identification and production of crop genotypes that stay consistently low in acrylamide-forming potential through a range of environments and conditions, including storage, and the development of best crop management practice.[25]

Conditions and length of storage are clearly aspects of potato management that can be optimized to keep acrylamide-forming potential as low as possible, and nutrition is another, with nitrogen availability, for example, increasing the acrylamide-forming potential of most varieties but decreasing it in some, whereas sulfur application reduces glucose concentrations and mitigates the effect of high nitrogen application in some varieties.[26] In this study, the impact of a third aspect of potato management, irrigation, was assessed, and the related issue of the effect of severe drought stress was investigated. The results showed water availability to have profound effects on the free amino acid and sugar concentrations and acrylamide-forming potential of potatoes. Lack of irrigation in the field and severe drought stress imposed in a glasshouse both brought about significant but different changes in composition, and different genotypes were affected in dissimilar fashion by the same treatment, indicating that there is no single, unifying potato tuber drought stress response.

■ MATERIALS AND METHODS

Chemicals. Ethanol (95% v/v, analytical grade) (Thermo Fisher Scientific UK Ltd., Loughborough, UK), HCl (Corning Life Science; supplied by Sigma-Adrich Company Ltd., Poole, UK), and acrylamide-$^{13}C_3$ (Sigma-Adrich Co. Ltd., Poole, UK) were used. KOH for IC chromatography (Thermo Fisher Scientific UK Ltd.),

amino acid standards (Phenomenex, Torrence, CA, USA), isotopically labeled amino acids (Cambridge Isotope Laboratories, Inc., Andover, MA, USA), and helium (high purity) (BOC Industrial Gases, Sheffield, UK) were also acquired.

Commercial Potato Samples. Tubers from five varieties of potatoes (*S. tuberosum*) grown commercially in the United Kingdom in 2011 were provided by Higgins Group (Doncaster, UK). They came from adjacent irrigated and not-irrigated fields in Herefordshire and Shropshire in western England and from Norfolk in eastern England, water being supplied to the irrigated plants when required in the judgment of the farm manager. The potatoes were planted in April and harvested between late September and early November 2011. Nitrogen, phosphate, and potassium fertilizer was applied at levels recommended in the *Fertiliser Manual* (RB209),[27] which takes into account soil type and intrinsic soil nutrient levels. In all cases, irrigated and not-irrigated plots received the same fertilizer treatment. After harvest, the potatoes were kept in a commercial potato store at 8.5−9.5 °C, in line with normal UK practice, until analysis in January 2012. The varieties were Hermes, Markies, and Ramos, considered to be drought-tolerant, and Lady Claire and Saturna, which are drought-sensitive. Replication was derived from randomly sampling five tubers from each plot.

Glasshouse Experiment. A split-plot experiment in two blocks was set up in four deep troughs (2 m long × 0.625 m wide × 0.55 m deep), consisting of a two-variety (Saturna and Markies) by two-treatment (watered and drought-stressed) factorial. Two containers formed a block, one for watered and one for drought-stressed main-plot treatment. Each main plot was further divided into six split-plots, with three tubers of each of the two varieties being planted on February 16, 2012, one tuber per split-plot. All split-plots were sealed compartments in the container to maintain watered and drought-stressed conditions and prevent leakage between compartments. 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. The troughs were filled with compost (Rothamsted mixture, requiring no additional fertilizer) on top of a 2.5 cm layer of J. Arthur Bower's Hydroleca (lightweight clay aggregate beads; William Sinclair Horticulture Ltd., Lincoln, UK) to allow efficient drainage.

Water was supplied automatically through a drip-feed for 3 min per day from the day of planting. On April 25 (68 days after planting) the plants began to flower, a developmental change that coincides closely with tuber initiation. At this point, watering to the drought treatment plants was reduced to 1 min per day and again on May 18 (91 days after planting) to 1 min per week. On June 7 (111 days after planting) the supply to the watered plants was increased to 6 min per day, and on September 6 (202 days after planting) watering to all plants was ceased to encourage senescence. Leaf water potential at mid-day was measured in a pressure chamber (PMS Instrument Co., Corvallis, OR, USA) to ensure that the plants for which water was withheld were drought-stressed. The plants were harvested on September 27, 2012 (223 days after planting).

Free Amino Acid and Sugar Concentrations. Free amino acids and sugars were measured as described previously.[21] Flour was prepared from individual freeze-dried tubers, and free amino acids were derivatized and then analyzed by gas chromatography−mass spectrometry (GC-MS) using an Agilent 5975 system (Agilent, Santa Clara, CA, USA). Note that arginine cannot be measured using this system, whereas cysteine concentrations were too low to measure accurately. Sugar concentrations were measured using a Dionex ion chromatography system with a 250 × 4 mm Carbopac PA1 column (Dionex Corp., Sunnyvale, CA, USA), operated using Chromeleon software, also as described previously.[21]

Acrylamide Formation. Acrylamide was measured in cooked potato flour after heating to 160 °C for 20 min. The analysis was performed by liquid chromatography−tandem mass spectrometry (LC-MS/MS) using an Agilent 1200 high-performance liquid chromatography (HPLC) system with a 6410 triple-quadrupole mass spectrometer with electrospray ion source in positive ion mode, as previously described.[21]

Statistical Analyses. The method of Residual Maximum Likelihood (REML) as implemented in *GenStat* (16th ed., VSN International Ltd., Hemel Hempstead, UK) was used to fit a linear mixed model to the data on the $\log_e$ scale, this transformation ensuring constant variance across the variety by treatment combinations. The analysis tested (F tests) the main effects and interactions between factors. For the field experiment, these factors were variety nested in type (drought-tolerant and -sensitive) and irrigation/nonirrigation, whereas random effects of location had to be accounted for. In the glasshouse experiment, the factors were variety and treatment (drought-stressed or watered), with blocks, main-plots, and split-plots being the random effects. Significant ($p < 0.05$, F test) terms for inspection were disseminated by considering differences between means of biological interest in terms of the standard error of the difference (SED) values on the relevant degrees of freedom (df). The least significant difference (LSD) was therefore used to judge significance at the 5% level.

For further inspection of the data, Pearson's correlation coefficients (r) were calculated between all pairs of responses, for the full data set and also for data pertaining to the separate treatments (irrigated and not-irrigated; watered or drought-stressed). These correlations were tested for significance using F tests.

RESULTS AND DISCUSSION

Effect of Irrigation on Tuber Composition in Five Potato Varieties Grown on Commercial Farms in the United Kingdom in 2011. Potato varieties Ramos, Lady Claire, Saturna, Hermes, and Markies were grown in three regions of the United Kingdom, namely, Herefordshire and Shropshire in western England and Norfolk in eastern England, under irrigated and not-irrigated conditions. The meteorological data for the United Kingdom in 2011 are available from the UK Meteorological Office: http://www.metoffice.gov.uk/climate/uk/2011/. Notable features of that year for England were a very warm and dry spring (the mean temperature for April was 3.5 °C above average), giving way to a rather cool summer with rainfall close to the average, although regionally variable. Eastern England, including Norfolk, where the Markies samples came from, was very dry in the spring, but the dry spell broke in June.

Data were obtained from tuber samples on free amino acids (excluding arginine, which cannot be measured by the method used, and cysteine, which was present at concentrations lower than would be required to allow reliable measurement) and the sugars glucose, fructose, and sucrose. Total amino acid content was calculated along with the ratio of free asparagine to total free amino acid concentration, a parameter that was identified as potentially related to acrylamide formation in a study that modeled the kinetics of acrylamide formation in French fry production.[28] Total sugar content was also calculated along with the sum of glucose and fructose as reducing sugars (potatoes contain very little maltose, and this was not measured). Data on acrylamide formation in heated flour were also obtained for analysis. The complete data set is given in Supporting Information Tables S1−S3.

The data were subjected to REML analysis following a $\log_e$ transformation to ensure constant variance across the variety by treatment (irrigated versus not irrigated) combinations. The results are shown in Table 1. There was a significant interaction ($p < 0.05$, F test) between treatment and variety nested in type for proline, tryptophan, glucose, total reducing sugars, and acrylamide. There was also a significant ($p < 0.05$, F test) interaction of type with treatment for free glutamine and the ratio of free asparagine to total free amino acids, indicating that the drought-tolerant and -sensitive varieties responded differ-

Table 1. p Values Denoting Significance of Main Effects and Interactions of Treatment Factors in Linear Mixed Model (REML) Analyses of Measured Variables for Five Varieties of Potatoes Grown, with or without Irrigation, on Commercial Farms in the United Kingdom in 2011[a]

	type	treatment	type × variety	type × treatment	type × variety × treatment
amino acids					
alanine	<0.001	0.796	**0.004**	0.434	0.715
glycine	<0.001	0.867	**<0.001**	0.059	0.782
valine	**<0.001**	0.225	0.468	0.614	0.803
leucine	0.006	0.115	**0.003**	0.738	0.584
isoleucine	**<0.001**	0.407	0.106	0.682	0.656
threonine	<0.001	0.632	**0.005**	0.693	0.509
serine	<0.001	0.598	**<0.001**	0.302	0.885
γ-aminobutyric acid	0.712	0.834	**<0.001**	0.057	0.752
proline	<0.001	<0.001	<0.001	0.903	**<0.001**
asparagine	0.047	0.088	**0.014**	0.919	0.786
aspartic acid	0.123	0.165	**<0.001**	0.139	0.958
methionine	**<0.001**	0.828	0.067	0.762	0.630
glutamic acid	<0.001	0.513	**0.006**	0.557	0.374
phenylalanine	<0.001	0.187	**0.004**	0.198	0.316
glutamine	<0.001	0.350	**<0.001**	0.021	0.893
lysine	<0.001	0.830	**0.006**	0.568	0.201
tyrosine	0.001	0.485	**<0.001**	0.287	0.111
tryptophan	0.011	0.420	**<0.001**	0.643	<0.001
total amino acids	<0.001	0.180	**<0.001**	0.178	0.762
asparagine/total amino acids	<0.001	0.480	**<0.001**	**0.001**	0.104
sugars					
glucose	0.165	<0.001	<0.001	0.605	<0.001
fructose	<0.001	**0.001**	**<0.001**	0.577	0.064
sucrose	0.065	0.642	0.129	0.149	0.482
reducing sugars	<0.001	<0.001	<0.001	0.994	0.010
total sugars	0.288	0.586	0.057	0.091	0.147
acrylamide	0.017	<0.001	<0.001	0.485	**<0.001**

[a]Variety is nested within type (drought-tolerant and -sensitive), and the × indicates the interaction between the treatment factors type and variety, type and treatment (irrigated or not irrigated), and type, variety, and treatment. P values in bold indicate the significant ($p < 0.05$, F test) terms that were selected for inspection (significant values not in bold were superseded by more complex terms in the ANOVA).

ently to irrigation with respect to these parameters. There were significant ($p < 0.05$, F test) varietal differences, nested in type, for free alanine, glycine, leucine, threonine, serine, γ-aminobutyric acid (GABA), asparagine, aspartic acid, glutamic acid, phenylalanine, glutamine, lysine, tyrosine, total free amino acids, the ratio of free asparagine to total free amino acids, and fructose. There was also a main effect ($p = 0.001$, F test) of treatment for fructose and a main effect of type ($p < 0.001$, F test) for free valine, isoleucine, and methionine. The relevant means, standard error of the difference between means (SED) (df), and least significant difference (LSD) (5%) values for comparisons are given in Table 2.

Table 2a shows the relevant means for the different varieties nested in type. Saturna had significantly different ($p < 0.05$, LSD) and lower concentrations of free alanine, glycine, threonine, serine, GABA, glutamine, lysine, and total free amino acids than the other varieties. The drought-tolerant varieties generally had greater concentrations of most free

Table 2. Comparison of Means for Free Amino Acids, Sugars and Acrylamide Formation for Five Varieties of Potatoes Grown, with and without Irrigation, on Commercial Farms in the UK in 2011

(a) Log$_e$ Means (n = 10), Standard Error of the Difference (SED), Degrees of Freedom (df), and Least Significant Difference (LSD) at the 5% Level of Significance for Comparison of Means for Variables with Only a Main Effect ($p < 0.05$, F Test) of Variety Nested within Type (Drought-Tolerant and -Sensitive)[a]

| | type × variety | | | | | | |
| | sensitive | | tolerant | | | | |
	Lady Claire	Saturna	Hermes	Markies	Ramos	SED/SE (df)	LSD (5%)
amino acids							
alanine	0.986 (2.680)	−0.029 (0.971)	1.922 (6.835)	1.804 (6.074)	1.898 (6.673)	0.2562 (40)	0.5179
glycine	0.042 (1.043)	−0.737 (0.479)	0.390 (1.477)	0.913 (2.492)	0.895 (2.447)	0.1796 (40)	0.3629
leucine	−0.017 (0.983)	−0.070 (0.932)	0.708 (2.030)	0.051 (1.052)	0.107 (1.113)	0.1789 (40)	0.3617
threonine	1.021 (2.776)	0.422 (1.525)	1.657 (5.244)	1.822 (6.184)	1.560 (4.759)	0.1684 (40)	0.3404
serine	1.460 (4.306)	0.368 (1.445)	1.661 (5.265)	2.020 (7.538)	1.944 (6.987)	0.2136 (40)	0.4317
γ-aminobutyric acid	3.137 (23.03)	1.367 (3.924)	2.216 (9.171)	2.259 (9.574)	2.134 (8.449)	0.2022 (40)	0.4087
asparagine	4.729 (113.18)	4.563 (95.87)	4.613 (100.79)	5.049 (155.87)	4.811 (122.85)	0.1347 (40)	0.2722
aspartic acid	3.261 (26.08)	2.772 (15.99)	2.946 (19.03)	3.204 (24.63)	3.281 (26.60)	0.1247 (40)	0.2520
glutamic acid	2.564 (12.99)	2.316 (10.14)	2.648 (14.13)	3.149 (23.31)	2.739 (15.47)	0.1550 (40)	0.3132
phenylalanine	0.366 (1.442)	0.449 (1.567)	1.381 (3.979)	0.556 (1.744)	1.027 (2.793)	0.2118 (40)	0.4281
glutamine	4.401 (81.53)	3.127 (22.81)	4.032 (56.37)	4.380 (79.84)	4.398 (81.29)	0.1747 (40)	0.3530
lysine	−0.030 (0.970)	−1.310 (0.270)	0.380 (1.462)	0.060 (1.062)	0.130 (1.139)	0.3450 (40)	0.6980
tyrosine	−1.287 (0.276)	−1.023 (0.360)	0.295 (1.343)	−0.808 (0.446)	−0.975 (0.377)	0.2949 (40)	0.5961
total amino acids	5.717 (303.99)	5.117 (166.83)	5.538 (254.17)	5.898 (364.31)	5.862 (351.43)	0.1415 (40)	0.2860
asparagine/total amino acids	−0.988 (0.372)	−0.554 (0.575)	−0.924 (0.397)	−0.848 (0.428)	−1.052 (0.349)	0.0593 (40)	0.1198
sugars							
fructose	−5.069 (6)	−4.060 (17)	−4.072 (17)	−3.405 (48)	−3.378 (34)	0.2846 (39)	0.5756
sucrose					−0.436 (647)	0.034 (39)	
total sugars					−0.343 (710)	0.033 (39)	

(b) Log$_e$ Means, Number of Observations in Means, Standard Error of the Difference (SED), Degrees of Freedom (df), and Least Significant Difference (LSD) at the 5% Level of Significance for Comparison of Means for Variables with a Type ($p < 0.05$, F Test) Effect Only

| | type | | | |
amino acid	sensitive	tolerant	SED (df)	LSD (5%)
valine	1.546, 20 (4.693)	2.316, 30 (10.14)	0.1266 (40)	0.2559
isoleucine	0.212, 20 (1.236)	0.978, 30 (2.659)	0.1273 (40)	0.2573
methionine	−0.072, 20 (0.931)	1.122, 30 (3.071)	0.1352 (40)	0.2733

(c) Log$_e$ Means, Number of Observations in Means, Standard Error of the Difference (SED), Degrees of Freedom (df), and Least Significant Difference (LSD) at the 5% Level of Significance for Comparison of Means for Variables with a Type (Drought-Tolerant and -Sensitive) by Treatment ($p < 0.05$, F Test) Effect

| | treatment | | | | | |
| | irrigated | | not irrigated | | | |
amino acid	sensitive	tolerant	sensitive	tolerant	SED (df)	LSD (5%)
glutamine	3.979, 10 (53.46)	4.214, 15 (67.63)	3.550, 10 (34.81)	4.326, 15 (75.64)	0.1747[b] (40)	0.3530
					0.1595[c] (40)	0.3223
					0.1426[d] (40)	0.2882
asparagine/total amino acids	−0.865, 10 (0.421)	−0.901, 15 (0.406)	−0.677, 10 (0.508)	−0.982, 15 (0.375)	0.0593[b] (40)	0.1198
					0.0541[c] (40)	0.1094
					0.0484[d] (40)	0.0978

(d) Log$_e$ Means (n = 5), Standard Error of the Difference (SED), Degrees of Freedom (df), and Least Significant Difference (LSD) at the 5% Level of Significance for Comparison of Means for Variables with Significant Interaction of Treatment with Variety Nested within Type (Drought-Tolerant and -Sensitive) ($p < 0.05$, F Test)

variety	treatment	proline	tryptophan	glucose	reducing sugars	acrylamide
Lady Claire[e]	irrigated	1.343 (3.831)	−0.070 (0.932)	−3.888 (20)	−3.510 (30)	0.557 (1.745)
Saturna[e]	irrigated	0.625 (1.868)	−3.140 (0.043)	−2.865 (57)	−2.493 (83)	1.156 (3.177)
Hermes	irrigated	1.777 (5.912)	−1.110 (0.330)	−3.310 (37)	−2.862 (57)	0.726 (2.067)
Markies	irrigated	2.846 (17.22)	−0.800 (0.449)	−4.135 (16)	−2.803 (61)	0.758 (2.134)
Ramos	irrigated	1.950 (7.029)	−1.510 (0.221)	−2.399 (91)	−1.789 (167)	0.873 (2.394)
Lady Claire[e]	not irrigated	3.062 (21.37)	−1.880 (0.153)	−4.459 (12)	−4.065 (17)	0.482 (1.619)
Saturna[e]	not irrigated	0.793 (2.210)	−1.910 (0.148)	−3.753 (23)	−3.339 (35)	0.852 (2.344)
Hermes	not irrigated	1.976 (7.214)	−0.780 (0.458)	−3.658 (26)	−3.210 (40)	0.678 (1.970)
Markies	not irrigated	2.925 (18.63)	−1.330 (0.264)	−4.025 (18)	−2.956 (52)	0.700 (2.014)
Ramos	not irrigated	4.650 (104.58)	−1.570 (0.208)	−3.992 (18)	−3.384 (34)	0.244 (1.276)

Table 2. continued

(d) Log_e Means ($n = 5$), Standard Error of the Difference (SED), Degrees of Freedom (df), and Least Significant Difference (LSD) at the 5% Level of Significance for Comparison of Means for Variables with Significant Interaction of Treatment with Variety Nested within Type (Drought-Tolerant and -Sensitive) ($p < 0.05$, F Test)					
variety	treatment	proline	tryptophan	glucose	reducing sugars	acrylamide
SED (df)		0.4358 (40)	0.4670 (40)	0.2425 (39)	0.3109 (39)	0.0863 (40)
LSD (5%)		0.8808	0.9440	0.4905	0.6288	0.1745

[a]The grand mean ($n = 50$) and corresponding standard error (SE) are given for sucrose and total sugars, these having no effects at all. Back-transformed means (mmol per kg for amino acids and mg per kg for sugars) are given in parentheses. [b]Comparing means having 10 replicates. [c]Comparing means having 10 replicates with means having 15 replicates. [d]Comparing means having 15 replicates. [e]Drought-sensitive varieties.

amino acids than the drought-sensitive varieties and were more consistent as a group, although Hermes had significantly different ($p < 0.05$, LSD) and lower free glycine, aspartic acid, and total free amino acids and greater leucine and tyrosine than Markies and Ramos. Markies had the highest concentration of free asparagine, significantly different ($p < 0.05$, LSD) from Lady Claire, Saturna, and Hermes but not from Ramos. However, Saturna had the greatest ratio of free asparagine to total free amino acids, significantly different ($p < 0.05$, LSD) from the other varieties. Markies and Ramos had the greatest fructose, significantly different ($p < 0.05$, LSD) from the other three varieties.

The two types (drought-tolerant and -sensitive) differed significantly ($p < 0.05$, F test) as a whole, regardless of within-type (varietal) influence, only in that the tolerant varieties showed greater concentrations of valine, isoleucine, and methionine (Table 2b), but there was an interaction between type and treatment (Table 2c): free glutamine was significantly different ($p < 0.05$, LSD) and lower in the not-irrigated samples than the irrigated for the drought-sensitive varieties but higher for the drought-tolerant varieties, whereas the ratio of free asparagine to total free amino acids was significantly different ($p < 0.05$, LSD) and higher in the not-irrigated samples than the irrigated samples for the drought-sensitive varieties but slightly lower for the drought-tolerant varieties.

There were more effects from the interaction between treatment and variety nested in type (Table 2d), indicating that the varieties responded differently to irrigation. There was a higher concentration of proline in the not-irrigated conditions for all varieties but significantly so ($p < 0.05$, LSD) for Lady Claire and Ramos, the latter having a tremendous increase of 13.9-fold from 7.02 mmol/kg with irrigation to 104.58 mmol/kg without irrigation. An increase in proline concentration in response to drought and other treatments that cause osmotic stress has been observed in many plant species and has led to the hypothesis that it is not just a symptom of stress but part of the stress response, decreasing cell osmotic potential and thereby increasing turgor while decreasing plant water potential. This hypothesis has been reviewed in detail (and with some skepticism) by Lawlor.[29] The results of the present study would support an increase in free proline concentration being part of the response to drought stress, except that the varietal type was not well-associated with the quantity of increased proline, because Lady Claire is known to be drought-sensitive, whereas Ramos is drought-tolerant. The tryptophan concentration also suggested a differential response to irrigation for the varieties: the concentration was significantly different ($p < 0.05$, LSD) and higher for Saturna in the not-irrigated than in the irrigated sample, but significantly different ($p < 0.05$, LSD) and lower for Lady Claire.

Drought stress has also been shown to bring about an interconversion of simple sugars and complex carbohydrates,

such as starch and fructan, in some plant species, and, as with proline, an increase in the concentration of simple sugars has been proposed to act as an osmotic protectant.[2,5] However, in this study, the glucose concentration was significantly different ($p < 0.05$, LSD) and lower in the not-irrigated samples for Lady Claire, Saturna, and Ramos and also substantially lower for Hermes, although slightly increased for Markies. Similar results were seen for total reducing sugars, albeit with Markies now showing a decrease.

There was no significant effect of the treatment, either on its own or interacting with type or variety nested in type, on the concentration of the other acrylamide precursor, free asparagine ($p = 0.088$, F test), but the indication was for an increase in all five varieties, with 124.84 mmol/kg in the not-irrigated samples and 107.66 mmol/kg in the irrigated samples, a 1.16-fold difference.

Acrylamide formation was lower for all varieties without irrigation and significantly so ($p < 0.05$, LSD) for Saturna (by 0.262 mg/kg on average) and Ramos (by 1.118 mg/kg on average). Previous studies have shown the relationship between precursor concentration and acrylamide formation in potato to be complex,[30] but glucose concentration has been a major factor in determining the acrylamide-forming potential in most of the data sets that have been obtained.[21,26,28,30−32] The correlation ($r = 0.625$, $p < 0.001$) between glucose concentration and acrylamide formation in this data set is shown in Figure 1A, and the lower glucose concentration in the not-irrigated samples was almost certainly responsible for the reduced acrylamide formation observed (Table 2d). There were also positive although not strong correlations with sucrose ($r = 0.304$, $p = 0.034$), total reducing sugars ($r = 0.492$, $p < 0.001$), and total sugar ($r = 0.458$, $p = 0.001$).

Free asparagine concentration has also been shown to contribute positively to the variance in acrylamide formation in some data sets,[21,30,33,34] but the correlation here was weak and negative ($r = −0.328$, $p = 0.022$). There was, however, a striking nonlinear relationship ($r = −0.589$, $p < 0.001$) of decreased acrylamide with increased proline (Figure 1B), with the very high proline concentrations brought about by nonirrigation, particularly in Ramos (Table 2d), being associated with reduced acrylamide formation. Proline has been shown to inhibit acrylamide formation in model systems[35] but is usually present in potatoes at much lower concentrations than asparagine, so the data presented here are the first to suggest that this could occur in a food matrix.

Effect of Drought Stress on Tuber Composition in Two Potato Varieties Grown in a Glasshouse. To apply a more controlled and severe drought stress, an experiment was conducted in a glasshouse. Two varieties, Markies, which is drought-tolerant, and Saturna, which is drought-sensitive, were selected for the study. For the plants to be able to develop a full-size root system and form tubers without physical
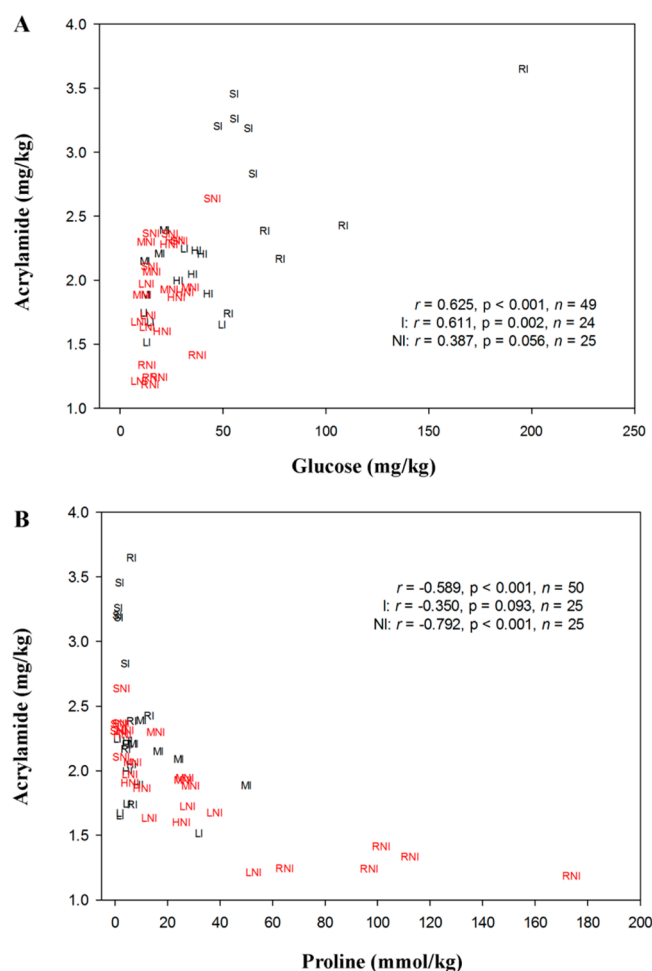
Figure 1. Graphs showing the correlation between glucose (A) and proline (B) concentrations and acrylamide formation in potato flour heated to 160 °C for 20 min. The flour was prepared from five varieties of potatoes grown, with and without irrigation, on commercial farms in the United Kingdom in 2011. The varieties were Hermes (H), Lady Claire (L), Markies (M), Ramos (R), and Saturna (S). Points on the graphs from irrigated potatoes are denoted I in black, whereas those for not-irrigated potatoes are denoted NI in red. The Pearson correlation coefficients (r), corresponding p values (F test), and number of pairs of observations (n) are given on the graphs.

restriction, the plants were grown in deep troughs filled with compost over a 2.5 cm layer of beads to allow drainage. A randomized, split-plot design was used, and drought stress was applied by reducing watering to half of the plants (drought) and maintaining it for the others (watered) (see Materials and Methods for details).

Watering of the drought plants was reduced after 68 days, at which point the plants were beginning to tuberize, and tubers were harvested after 7 months, when the plants were beginning to senesce. In previous glasshouse experiments on potato, the life cycle from planting to harvest has been 12 weeks,[34] the difference being that the plants were grown in pots containing vermiculite so that feeding with minerals could be controlled. In the present study, growing the plants in sufficient depth of compost to allow full root development resulted in a life cycle duration much more similar to that in the field in the United Kingdom.

Leaf water potential (LWP) at mid-day was monitored to ensure that the plants for which water was being withheld were drought-stressed. At the beginning of the treatment, LWP ranged from 4 to 6 MPa for Saturna and from 6 to 7.5 MPa for Markies. After 3 weeks of the treatment, the range of LWP for watered Saturna was 4.5–5.5 MPa and for watered Markies, 3.5–5.5 MPa, whereas the range in drought-stressed Saturna had risen to 8.5–10.5 MPa and for Markies, 10–11 MPa. These levels were maintained until the plants began to senesce.

Data were obtained on concentrations of free amino acids (once again without arginine and cysteine) and sugars (glucose, fructose, and sucrose). Total free amino acid content was calculated along with the ratio of free asparagine to total free amino acid content. Total sugar content was also calculated along with the sum of glucose and fructose as reducing sugars. Data on acrylamide formation in heated flour were also obtained for analysis. The full data set is given in Supporting Information Tables S4–S6.

REML was applied to the data on the $\log_e$ scale, this transformation ensuring constant variance across the variety by treatment combinations. The results are given in Table 3.

Table 3. p Values Denoting Significance of Main Effects of Drought Stress and Variety and Interactions between the Two in Linear Mixed Model (REML) Analyses of Measured Variables for Two Varieties of Potato Grown in a Glasshouse with Water Provided or Withheld[a]

	stress	variety	stress × variety
amino acids			
alanine	0.345	**0.011**	0.775
glycine	0.178	**0.005**	0.869
α-aminobutyric acid	**0.007**	0.926	0.362
valine	0.853	0.116	**0.028**
leucine	0.823	0.001	**0.005**
isoleucine	0.916	**0.007**	0.087
threonine	0.185	0.116	0.878
serine	0.469	0.231	0.728
γ-aminobutyric acid	0.092	0.222	0.826
proline	0.238	**0.025**	0.159
asparagine	**0.036**	0.849	0.953
aspartic acid	0.239	0.520	0.709
methionine	0.984	0.123	0.333
glutamic acid	0.301	0.213	0.234
phenylalanine	0.937	**<0.001**	0.067
glutamine	0.822	**0.004**	0.937
ornithine	0.074	0.071	0.417
lysine	0.911	**<0.001**	0.079
histidine	0.209	0.012	**0.020**
tyrosine	0.885	**<0.001**	**0.019**
tryptophan	0.795	**0.018**	0.158
total amino acids	0.062	0.222	0.884
asparagine/total amino acids	0.249	**0.007**	0.531
sugars			
glucose	0.534	**0.013**	0.297
fructose	0.963	0.813	0.457
sucrose	0.240	0.220	0.859
reducing sugars	0.391	**0.021**	0.376
total sugars	0.266	0.146	0.965
acrylamide	0.178	0.257	0.176

[a]The × indicates the interaction between the factors: drought stress (stress) and variety. p values in bold indicate the significant ($p < 0.05$, F test) terms for inspection.

Table 4. Comparison of Means for Free Amino Acids, Sugars and Acrylamide Formation for Two Varieties of Potato Grown in a Glasshouse with Water Provided (Watered) Or Withheld (Drought)

(a) Log_e Means ($n = 12$), Standard Error of the Difference (SED), Degrees of Freedom (df), and Least Significant Difference (LSD) at the 5% Level of Significance for Comparison of Means for Free Amino Acids and Sugars with Only a Main Effect ($p < 0.05$, F Test) of Variety[a]

	variety			
	Markies	Saturna	SED/SE (df)	LSD (5%)
amino acids				
alanine	2.63 (13.87)	1.94 (6.96)	0.231 (13)	0.499
glycine	1.85 (6.36)	1.17 (3.22)	0.206 (13)	0.466
isoleucine	0.96 (2.61)	1.95 (7.03)	0.311 (13)	0.673
threonine	1.89 (6.62)		0.099 (13)	
serine	2.68 (14.60)		0.108 (13)	
γ-aminobutyric acid	3.29 (26.76)		0.075 (13)	
proline	3.78 (43.82)	2.90 (18.17)	0.347 (13)	0.750
aspartic acid	3.34 (28.16)		0.100 (13)	
methionine	0.82 (2.27)		0.131 (13)	
glutamic acid	0.660 (1.93)		0.117 (13)	
phenylalanine	−0.07 (0.93)	1.56 (4.76)	0.368 (13)	0.795
glutamine	4.54 (93.69)	3.82 (45.60)	0.206 (13)	0.446
ornithine	0.22 (1.25)		0.164 (13)	
lysine	1.43 (4.18)	2.12 (8.31)	0.161 (13)	0.348
tryptophan	−1.30 (0.27)	0.03 (1.03)	0.487 (12)	1.060
total amino acids	6.18 (482.99)		0.065 (10)	
asparagine/total free amino acids	−1.06 (0.35)	−0.91 (0.40)	0.046 (10)	0.103
sugars				
glucose	5.44 (230.44)	4.68 (107.77)	0.266 (13)	0.574
fructose	3.62 (37.34)		0.159 (13)	
sucrose	7.14 (1261.43)		0.147 (13)	
reducing sugars	5.65 (284.29)	5.01 (149.90)	0.244 (13)	0.528
total sugars	7.30 (1480.30)		0.141 (13)	

(b) Log_e means ($n = 12$), Standard Error of the Difference (SED), Degrees of Freedom (df), and Least Significant Difference (LSD) at the 5% Level of Significance for Comparison of Means for Amino Acids and Sugars with Only a Main Effect ($p < 0.05$, F Test) of Stress[b]

	treatment			
amino acid	drought	watered	SED (df)	LSD (5%)
α-aminobutyric acid	−1.00 (0.37)	−1.19 (0.30)	0.002 (1)	0.027
asparagine	5.49 (242.26)	4.88 (132.03)	0.035 (1)	0.442

(c) Log_e Means ($n = 6$), Standard Error of the Difference (SED), Degrees of Freedom (df), and Least Significant Difference (LSD) at the 5% Level of Significance for Comparison of Means for Amino Acids and Sugars with an Interaction ($p < 0.05$, F Test) between Variety and Stress[c]

	treatment					
	Markies		Saturna			
amino acid	drought	watered	drought	watered	SEC (df)	LSD (5%)
valine	2.36 (10.59)	2.92 (18.54)	3.36 (28.79)	2.73 (15.33)	0.338 (13)	0.731
					0.288 (7)	0.670
leucine	0.27 (1.31)	0.75 (2.12)	1.87 (6.49)	0.92 (2.51)	0.300 (13)	0.648
					0.846 (1)	8.108
histidine	1.04 (2.83)	0.64 (1.90)	2.16 (8.67)	0.69 (1.99)	0.288 (13)	0.662
					0.378 (2)	1.665
tyrosine	−1.61 (0.20)	−0.85 (0.43)	1.29 (3.63)	0.08 (1.08)	0.519 (13)	1.122
					1.296 (1)	11.543

(d) Log_e Means ($n = 6$) for Acrylamide, Glucose, Fructose, Sucrose, Reducing Sugars, and Total Sugars[d]

	treatment				
	Markies		Saturna		
response	drought	watered	drought	watered	grand mean, SE
acrylamide	7.534 (1871)	7.954 (2847)	7.573 (1945)	7.535 (1872)	7.649 (2099), 0.090
glucose	5.780 (324)	5.110 (166)	4.730 (113)	4.630 (103)	5.060 (158), 0.149
fructose	3.560 (35)	3.750 (43)	3.710 (41)	3.460 (32)	3.620 (37), 0.159
sucrose	7.510 (1826)	7.100 (1212)	7.22 (1366)	6.72 (829)	7.140 (1261), 0.147
reducing sugars	5.930 (376)	5.380 (217)	5.060 (158)	4.960 (143)	5.330 (206), 0.137
total sugars	7.700 (2208)	7.290 (1466)	7.330 (1525)	6.890 (982)	7.300 (1480), 0.141

Table 4. continued

[a]The grand mean ($n = 24$) and corresponding standard error (SE) are given for amino acids and sugars with no effects of variety or stress. Back-transformed means (mmol per kg for amino acids and mg per kg for sugars) are given in parentheses. [b]Back-transformed means (mmol per kg) are given in parentheses. [c]The first SED and LSD of each pair in the table are for comparison of the varieties in either drought or control condition; the second SED and LSD of each pair are for all other comparisons. Back-transformed means (mmol per kg) are given in parentheses. [d]There was no significant ($p < 0.05$, F test) effect of stress for acrylamide or sugars (Table 3), but the full means table is included here for inspection. Back-transformed means (mg per kg for sugars and μg per kg for acrylamide) are given in parentheses.

There was an interaction ($p < 0.05$, F test) between stress and variety for free valine, leucine, histidine, and tyrosine. There was a main effect ($p < 0.05$, F test) of stress only for α-aminobutyric acid (AABA) and asparagine. There was a main effect ($p < 0.05$, F test) of variety only for free alanine, glycine, isoleucine, proline, phenylalanine, glutamine, lysine, trypto-phan, the ratio of free asparagine to total free amino acids, glucose, and reducing sugars. There was no significant ($p < 0.05$, F test) effect of either stress or variety for acrylamide.

The relevant means and SED (df) and LSD (5%) values for metabolites showing a main effect of variety only are given in Table 4a. Markies had significantly different ($p < 0.05$, F test) and greater concentrations of free alanine, glycine, proline, and glutamine, as well as glucose and reducing sugars, than Saturna. Meanwhile, Saturna had significantly different ($p < 0.05$, F test) and greater concentrations of free isoleucine, phenylalanine, lysine, and tryptophan and a greater ratio of free asparagine to total free amino acids than Markies.

Two free amino acids, asparagine and AABA, showed a significant effect ($p < 0.05$, F test) of the treatment alone (in other words, the two varieties responded in similar fashion) (Table 4b). AABA concentration was somewhat greater in the potatoes from drought-stressed than watered plants, but free asparagine was almost doubled, from 132.03 to 242.26 mmol/kg. Four other free amino acids, valine, leucine, histidine, and tyrosine, showed an effect of the stress interacting with variety (in other words, the varieties responded differently) (Table 4c). For free valine, Saturna showed a substantial increase under the stress, whereas Markies had a considerable decrease, so that the difference between the varieties was significant ($p < 0.05$, LSD) under stress. A similar effect was seen for leucine and tyrosine. For histidine there was an increase under the stress for both varieties, but more so for Markies (4.4-fold), giving a significant ($p < 0.05$, LSD) difference between the varieties under the stress condition.

Proline concentration increased in both varieties in response to drought but, in contrast to the field-grown potatoes, the response was not statistically significant ($p = 0.238$, F test, for the main effect of stress and $p = 0.159$, F test, for the variety by stress interaction). There was also no overall significant effect of the treatment on sugar concentrations or acrylamide formation, but Table 4d gives the means for information. Markies showed considerably less acrylamide in the drought than in the watered condition (consistent with the result of the field study), whereas Saturna showed slightly more. For Saturna, the increase in acrylamide formation could be explained by increases in glucose and fructose. However, in Markies glucose was substantially higher in the drought samples than in the watered samples (Table 4d), and, given that free asparagine concentration was also almost doubled in the drought-stressed samples compared with the watered (Table 4b), the reduction in acrylamide formation is surprising. Fructose concentration did decrease in drought-stressed Markies, and fructose has been shown to be more reactive than glucose in kinetic modeling of

acrylamide formation in French fries.[28] Nevertheless, the fact that both total reducing sugar and free asparagine concentration rose in Markies in response to drought stress but acrylamide formation declined does suggest that other factors were involved, and this requires further study.

Another notable aspect of the study was that lack of irrigation to commercial, field-grown potatoes, destined for the food chain, resulted in statistically significant changes in composi-tion, even in the temperate United Kingdom in a year with not-unusual levels of rainfall. This is an example of the potential impact of environmental and management factors on crop composition, nutritional value, and processing properties.[2] Furthermore, the changes caused by lack of irrigation in the field were different in some respects from those brought about by more extreme drought stress imposed in the glasshouse. The most striking contrast between the effects of the moderate and more extreme stress was in the change in free asparagine concentration, which did not differ with statistical significance in the irrigated versus not-irrigated field samples but which showed a big increase in response to drought stress in the glasshouse.

The accumulation of free asparagine has been observed in diverse plant species in response to a range of abiotic stresses,[36] and, of course, this has potential implications for food safety.[2] Free proline, an increase in the concentration of which is also associated with abiotic stress, did not increase significantly in response to drought in the glasshouse experiment, but did in the field. Proline has been shown to increase in concentration in the leaves of drought-stressed potato plants,[37,38] with more accumulating in drought-sensitive than -tolerant genotypes. However, drought tolerance has also been suggested to be dependent on root growth rather than responses in the leaves.[39] The subject is reviewed comprehensively by Monneveux et al.,[40] but, as is generally the case with effects of stresses such as drought on plants, physiological studies have focused on the vegetative parts of the plant and the ability of the plant to survive the stress, not on the composition of the tubers. Unfortunately, the field data on Ramos, which showed a massive change in proline concentration in tubers in response to lack of irrigation, was not available in time to have included this variety in the glasshouse experiment. The increase in proline concentration in Ramos in the field was far greater than in the other varieties, showing that different genotypes of potato are affected in dissimilar fashion by the same abiotic stress. Consequently, the study did not identify a unifying potato tuber drought stress response.

The differing responses of the potatoes to lack of irrigation in the field and severe drought stress imposed in a glasshouse support the growing realization among plant physiologists that mild and extreme stresses may provoke very different responses in plants. This was discussed by Hancock et al.[41] in the context of temperature stress responses in potato, drawing on hypotheses put forward by Skirycz et al.[42] and Cramer et

al.[43] The results of the present study are consistent with this new paradigm.

Implications for Commercial Potato Production. Although the field and glasshouse experiments produced different responses and raised new questions about the relationship between precursor concentration and acrylamide formation, the effect of irrigation in the field on acrylamide formation during processing was clear: irrigation led to an increase in acrylamide formation. It must be borne in mind that the study concerned samples from one harvest year. Nevertheless, it would be sensible for potato farmers to irrigate only if necessary to maintain the health and yield of the crop.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Free amino acid and sugar concentrations and acrylamide formed in heated flour. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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