Kinetic Modelling of Acrylamide Formation during the Frying of Potato Chips

Matthew Knight[2], Simon McWilliam[1], Sarah Peck[1], Georgios Koutsidis[2*], Gemma Chope[1], Ian Puddephat[1]and Bronislaw Wedzicha[3]

[1] PepsiCo International Ltd, Beaumont Park, 4 Leycroft Road, Leicestershire, LE4 1ET, UK

[2] Department of Applied Sciences, University of Northumbria at Newcastle, Newcastle City Campus, Ellison Place, Newcastle upon Tyne, Tyne and Wear, NE1 8ST

[3] School of Food Science and Nutrition, University of Leeds, Leeds LS2 9JT

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*Corresponding author at: Department of Applied Sciences, University of Northumbria at Newcastle, Newcastle City Campus, Ellison Place, Newcastle upon Tyne, Tyne and Wear, NE1 8ST, UK.

Tel.: +44 191 227 3978, E-mail address: Georgios.koutsidis@northumbria.ac.uk

ABSTRACT: The effect of potato tuber composition, frying time and temperature on acrylamide formation in potato chips was investigated and a mathematical model of the kinetics of acrylamide formation is provided. Moisture-temperature-time profiles were obtained for potato slices during frying to enable the determination of the 'effective' reaction time by identifying the critical moisture content (6% dwb) for acrylamide formation to commence and using dehydration curves to calculate subsequent frying time to finished product moisture-content. The chemical kinetic model conformed to the following rate equation over a one hundred-fold range of acrylamide concentrations:

$$\frac{d[acryl]}{dt} = k_1[glucose][asn] + k_6[fructose]\frac{[asn]}{[TAA]}$$

where [TAA] represents total amino acid concentration. The timescale of the frying process meant that the chemical reactions were all in their initial rate phase. Kinetic parameters confirm that the fructose-dependent reaction (caramelization) contributes twice as much acrylamide as the reaction of glucose (Maillard reaction).

INTRODUCTION

Acrylamide is a very well-known process contaminant formed *via* the Maillard reaction between reducing sugars and/or carbonyl compounds and the amino acid asparagine (Mottram, Wedzicha, & Dodson, 2002) in several different foodstuffs including potato-based, cereal-based foods and coffee (Lineback, Coughlin, & Stadler, 2012). In potato-based products such as potato chips and French fries, the combination of the thermally intense frying process and the high native levels of asparagine may generate significant levels of acrylamide, 117–4,215 $\mu g\ kg^{-1}$ and 59–5,200 $\mu g\ kg^{-1}$ respectively (Lineback et al., 2012). Since the discovery of acrylamide in foods in 2002, food businesses and researchers worldwide have undertaken substantial research activities resulting in over 1,400 publications, investigating formation mechanisms, kinetics, occurrence and exposure, toxicology and mitigation strategies. Information were compiled into national databases, the FoodDrinkEurope acrylamide toolbox (currently in its 15th edition) and scientific opinion documents (EFSA CONTAM panel, 2015) leading to Commission Regulation (EU) 2017/2158 establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in foods. Amongst others, where possible, control and mitigation strategies proposed were the selection of raw materials based on asparagine and reducing sugar levels, re-evaluation of agronomy practices, processing and/or storage conditions of raw materials and reformulation strategies incorporating acrylamide mitigation agents. Mathematical models predicting acrylamide yields have also been proposed as a means to control and monitor acrylamide content in end products and a number of approaches have been followed including pattern recognition (Pedreschi, Segtnan, & Knutsen, 2010), artificial neural networks (Serpen & Gökmen, 2007) and kinetics (Parker, Balagiannis, Higley, Smith, Wedzicha, & Mottram, 2012). Such approaches are of significant value to the industry as they could provide predicting and monitoring capabilities in addition to the relatively laborious analytical determinations of acrylamide content. Moreover, associations between important quality parameters such as colour, end moisture and acrylamide could also be made, thus providing a useful quality control tool that links physicochemical properties of end-products with consumer safety. However, most pattern recognition and neural network approaches as well as simple colour-acrylamide or moisture-acrylamide kinetic models may require

frequent re-validation exercises in cases of changes in raw materials and/or process adjustment. The present study recognises the opportunity for a quantitative tool for potato crisp (PC) production using material composition, process conditions (temperature, time) and finished product parameters (*e.g.* moisture content) to predict acrylamide levels while allowing for variation in production practices. The hypothesis being tested is that a combination of systematic adjustment of potato slice-composition to achieve a wide a range of sugar and amino acid ratios coupled to precise control of process conditions in a bench top PC fryer will allow for the development of a robust yet simplified kinetic modelling of acrylamide formation specific to PC processing.

METHODS AND MATERIALS

Materials. Fresh potato tubers (cv. Lady Rosetta) used in the kinetic studies were sourced from two stocks from Leycroft Road Walkers Factory, Leicester, UK. Tubers (cv. Lady Rosetta) used for the determination of dehydration rates were sourced from two cultivation locations in the UK, specifically North Yorkshire and Norfolk, to provide a range of Dry Matter (DM) contents.

Chemicals. Glucose, fructose and sucrose were all sourced from Sigma-Aldrich Co., Ltd. (Poole, Dorset, U.K.) and were of >99% purity. Asparagine, glutamine and glycine were sourced from Sigma-Aldrich Co., Ltd. (Poole, Dorset, U.K.) and Fischer Scientific (Loughborough, Leicestershire, U.K.) and were of >97% purity.

Preparation of sugar- and amino acid- fortified potato slices for kinetic studies. Tubers were graded for size (producing PC with a diameter of 50-70 mm) and sliced using an industrial slicer to a standard PC thickness with a target coefficient of variation (CV) of <11%. The stock potato broth was made by adding 2.5 kg of fresh, washed and peeled potato slices to a freestanding heated water tank containing 25 L of water at 80 °C. The slices were placed inside a muslin sack and submerged in water for 1 min under continuous agitation to ensure equal and reproducible mass transfer (inter- and intra-cellular component leaching). The slices were then removed and discarded and the broth contained within the tank was kept at 80 °C. Fortification of sugars through blanching involved treatments with12 sugar solutions, namely; 0.2, 0.4, 0.6, 0.8 and 1.0% w/v glucose, 0.2, 0.4, 0.6, 0.8 and 1.0% w/v fructose and

68 0.2%/0.2% w/v glucose/fructose and finally 0.2%/0.2%/0.2% w/v glucose/fructose/sucrose.
69 Fortification of amino acids through blanching involved treatments with 10 amino acid solutions,
70 namely; 1.5, 2.0, 2.5, 3.0 and 3.5% w/v asparagine, 0.6% and 1.2% w/v glutamine and 0.5, 1.0 and
71 2.0% w/v glycine.

72 Sugar- or amino acid- fortified blanching broths (1.25 kg) were prepared in a metal bowl and transferred
73 to a water bath (Nickel Electro Ltd, Weston-super-Mare, UK) set at 80 °C. The temperature of the
74 fortified broths was measured using a handheld digital thermometer (RS Components Ltd., Northants,
75 UK). Upon reaching 78 °C, 50 potato slices were placed in the metal bowl and stirred continuously for
76 1 min. The blanched slices were transferred from the solution onto paper towels to remove the excess
77 liquid. Eighteen slices were taken, snap frozen in a liquid nitrogen cradle for 90 s, ground (for <15 s)
78 to a fine powder using a WSG 30K Spice Grinder (Conair Corp., Stamford, CT), placed in 150 mL
79 plastic sample pots and transferred to a -80 °C freezer until compositional determinations.

80 Blanched slices (n=27) were placed individually into a custom-built 27 cell fry basket. The fry basket
81 was reassembled and submerged into the deep fat fryer (Bartlett Yeoman, Yeoman) for pre-defined times
82 (30-180 s) and oil temperatures (145-165 °C) according to the experimental design. Temperature data
83 were recorded in each corner of the frying basket using a datalogger (Thermosense, Manchester, UK).
84 After frying, the PCs were removed from the oil and allowed to cool for 4-5 min before being placed
85 into a -20 °C freezer for storage, pending analysis. The approach resulted in 480 kinetic runs across 4
86 temperatures and 80 potato slice compositions.

87 **Determination of dehydration curves during PC frying.** The effect of the dry matter-content (DM)
88 on dehydration rates during frying was examined using a factorial experimental design of target
89 temperature (150-190 °C), across three categories of DM (21, 23 and 25%, (sd = ± 0.33)) and time (54-
90 200 s), comprising of 50 experimental conditions (n=27 slices for each condition) using Design Expert
91 software. Two batches of Lady Rosetta potatoes were sorted and graded to include tubers in the 50-
92 70 mm range from rose to heel. Each tuber was weighed, both in air and in water and the dry-matter
93 content for each potato was calculated. Tubers for each category of DM were rumble-peeled and washed
94 before being sliced to the standard PC thickness. The slices were rinsed briefly in cold water, surface

95 water was removed by patting them dry with a paper towel and subsequently fried as determined by the
96 experimental design. The procedure for frying, cooling and storage was identical to that detailed above.

97 **Analysis of sugars by Ion Chromatography (IC).** Aliquots of snap-frozen, homogenised potatoes
98 (8.5±0.1 g) were weighed into 50 mL centrifuge tubes, 20 mL of UHP Water (18.2 Ω) were added and
99 the samples were mixed thoroughly by inversion. The tubes were subsequently vortexed for 1 min, the
100 contents left to settle for 5 min and centrifuged at 4500 rpm for 2 min at 8 °C. Ethanol (3 mL, 96%)
101 was added to 15 mL centrifuge tubes followed by 1 mL of the supernatant and the samples were mixed.
102 The addition of sample to the ethanol took place within 15 min of the sample being weighed into the
103 50 mL centrifuge tube. Extracts were diluted (1:50 (v/v)) in UHP water and aliquots (20 µL) analysed
104 using a Dionex ICS 3000 system equipped with a CarboPac PA20 analytical column (3 mm x 150 mm)
105 and a CarboPac PA20 guard column (3 mm x 30 mm). The mobile phase was generated using an Elugen
106 III KOH cartridge. The eluent concentration was 15 mM at a flow rate of 0.37 mL/min and the oven
107 and detector temperature were set at 25 °C. A gold working electrode and pH Ag/AgCl combination
108 reference electrode were used in the carbohydrate setting EC waveform. The total run time of the
109 method was 22 min.

110 **Analysis of amino acids by GC-MS (EZ:Faast Method).** The EZ:Faast GC-MS kit was used for free
111 amino acid analysis as described previously (Elmore, Mottram, Muttucumaru, Dodson, Parry, &
112 Halford, 2007). Samples were analysed using an Agilent 5975 GC-MS equipped with a ZB-AAA
113 capillary column (10 m x 250 µm x 0.25 µm). Samples (2 µL) were injected at 250 °C in split mode
114 (10:1) using helium as a carrier gas at a flow rate of 1.1 mL/min. The initial oven temperature was set
115 at 110 °C and immediately followed by a temperature ramp to 320 °C at 30 °C /min. The transfer line,
116 ion source and quadrupole temperatures were set at 280 °C, 240 °C and 180 °C, respectively. The mass
117 spectrometer was operated in the total ion scan mode (*m/z* 45-450) and *m/z* 155 and 160 were used as
118 the quantitation ions for asparagine and ^{13}C-asparagine, respectively.

119 **Loss on drying.** Aliquots of snap-frozen, homogenised potato (2-3 g) were weighed into pre-dried,
120 cooled in a desiccator and accurately weighed stainless steel dishes. The samples were then placed in a

forced-air oven at 100±2 °C for 2 days, cooled in a desiccator, weighed and the % loss on drying calculated.

Determination of moisture-content. Moisture-content was determined gravimetrically on a pooled subsample of the fried product obtained according to the factorial experimental designs described previously. Samples were crushed, weighed accurately (1-2 g, 4 d.p.), placed in an industrial oven at 105 °C for 18 hours, cooled in a desiccator and reweighed.

Analysis of acrylamide by LC-MS/MS. Fried PC samples (25 g) were homogenised in a Robot Coupe food processor using 5 pulses. Homogenised samples (1.00±0.02 g) were weighed into a 12 mL centrifuge tube, internal standard solution (10 mL of 20 ppb $^{13}C_3$ acrylamide in HPLC grade methanol) was added and followed by vortex mixing. Samples were then placed on a shaker mixer for 20 min and subsequently centrifuged at 13500 rpm for 10 min at 25 °C. Automated purification of the crude extracts was performed by transferring aliquots (300 µL) to a primed 96 well Bond Elut plate (Agilent Technologies Inc., Santa Clara, United States) using a Hamilton STARlet robot (Hamilton Bonaduz AG, Bonaduz Switzerland) coupled to a Cerex 96 multichannel SPE Positive Pressure Processor (SPEware Corp, Baldwin Park, California, United States). Low pressure was applied to the plate 30 min after sample addition and for 5-10 s. UHP water (300 µL) was added into each sample well and left to stand for 15 min followed by a second low pressure application step for 5-10 s. Both the initial sample elution and wash were discarded and a fresh plate was used for the elution and collection of the purified extract using 150 µL of UHP water, followed by standing for 15 min and application of low pressure to the plate for 5-10 s until the plate filters were dry.

LC-MS/MS analyses were performed using an Applied Biosystems API 5000 LC/MS/MS system equipped with an Agilent 1200 HPLC pump and a Gerstel Multipurpose Sampler 2XL. Ten microliters of sample were injected on a Hypercarb column (100 mm x 3 mm, 5 µm, Thermo Scientific, Waltham, USA) thermostated at 60 °C. The mobile phase consisted of deionised water: methanol: formic acid 850:150:1.0 (v/v/v) with a flow rate of 0.25 mL/min. The following transitions were used for quantitation purposes: quantification ion 72→55, (declustering potential 55 V, collision energy 25 eV, collision cell exit potential 10 V), confirmation ion 72→44, (declustering potential 51.3 V, collision

148 energy 47 eV, collision cell exit potential 18.4 V), internal standard ion 75→58, (declustering potential
149 55 V, collision energy 25 eV, collision cell exit potential 10 V).

150 **Determination of extractable oil using the Soxhlet method.** Extractable oil of the PC samples was
151 determined using the Campden BRI method - TES-AC-536, which encompasses a Weibull-Stoldt
152 extraction (acid hydrolysis followed by Soxtec extraction) and crude fat (Soxtec) extraction.

153 **RESULTS AND DISCUSSION**

154 **Infusion Blanching Fortification of Potato Slices.** The objective was an experimental system which
155 allowed for the tuber sugar and amino acid composition to be varied independently without the
156 constraints of native composition. Glucose (0.2, 0.4, 0.6, 0.8, 1.0% w/v), fructose (0.2, 0.4, 0.6, 0.8,
157 1.0% w/v) and sucrose (0.2% w/v) fortified broths allowed for the study of their individual roles in
158 acrylamide formation. Similarly, asparagine, glutamine and glycine broths allowed for the study of
159 reactions related to both acrylamide formation (asparagine) as well as competing Maillard reaction
160 pathways (glutamine and glycine). Additionally, fortification of the potato samples with different
161 amounts of amino acids relative to asparagine allowed the examination of the effect of the ratio of
162 asparagine: total amino acid which has been shown to correlate to acrylamide formation in potato-based
163 systems, under low sugar conditions (Elmore et al., 2007). Linear relationships between the
164 concentration of the fortifying solute(s) in the blanching broth and their concentration in the blanched
165 slice were observed on each occasion.

166 During blanching of potato slices, the high temperatures disrupt the cell membranes of the potato,
167 allowing components both from within the cell and in the broth to diffuse across the gradient (Arroqui,
168 Rumsey, Lopez, & Virseda, 2002; Bartlett, et al., 2020). To prevent excessive leaching of the native
169 components and, in turn, shift the mass transfer equilibrium, the blanching process utilised a 'broth'
170 made from blanched potato slices in the same potato: water ratio (1:10 w/v).

171 The fortification of slices with asparagine and glycine was conducted to achieve a substantial difference
172 in asparagine: total amino acid (Asn:TAA) ratios across the experimental design. The Asn:TAA ratios
173 ranged from 0.11 to 0.79; these limits were achieved by infusing in a 2.0% glycine and 3.5% asparagine

174 solution, respectively. The average ratio in blanched control samples was 0.36. A total of 480 kinetic
175 runs were obtained across 4 temperatures and 80 composition treatments. The infusion process resulted
176 in an average RSD of 20% for sugars and 24% for amino acids.

177 Previous studies had been concerned with correlation between the ratios of asparagine to total amino
178 acids and acrylamide formation in potato products (Becalski et al., 2004; Elmore et al., 2007). Elmore
179 et al. showed that when the sugar concentration of potato varieties is low, the level of asparagine relative
180 to the total amino acid content of the tuber becomes a driving factor in acrylamide formation. Low sugar
181 levels limit the formation of acrylamide and it is at such low levels that competition of asparagine with
182 other amino acids becomes most important. Becalski et al., on the other hand, did not find such
183 correlations when examining the three-dimensional relationship between Asn:TAA ratio, sugar
184 concentration and acrylamide yield; however, the authors utilised a range of 0.12-0.38 Asn:TAA, which
185 may be too narrow to identify a global trend that is also valid at high asparagine contents.

186 **PC dehydration rates.** Commercially produced PC have an average moisture content less than 2.0%
187 (Kita, Bråthen, Knutsen, & Wicklund, 2004; Wu, Jouhara, Tassou, & Karayiannis, 2012). Moisture
188 plays a critical role in acrylamide formation, with low moisture-high temperature conditions (roasting,
189 frying, baking) generating higher acrylamide yields (Wicklund, Ostlie, Lothe, & Kita, 2006). The
190 thermal input, specifically the heating time and temperature, is key for PC dehydration and the
191 formation of acrylamide (FoodDrinkEurope, 2019). The water loss associated with high temperature
192 treatments occurs primarily in the outer layer of foodstuff, such as the outer layer of French fries or
193 bread. It is suggested here that, owing to the potato slices being relatively thin, dehydration of PCs
194 occurs at an approximately constant rate throughout the slice during frying and, thus, acrylamide
195 formation is also deemed to be uniform across the entire slice.

196 The greatest uncertainty in the modelling of the kinetics of the frying process is the actual temperature
197 at which the reactions take place and the reaction time. The hypothesis being tested here is that the
198 formation of acrylamide begins when the moisture content of the PC slice falls to a 'critical' value and
199 since, at that time the slice would have lost a high proportion of its moisture, the reaction will occur
200 isothermally at, effectively, the final temperature of the frying oil. The reaction time would then be the

time from the start of the reaction to that required to achieve the final moisture content. The experimental plan was, therefore, to plot moisture-temperature-time relationships for potato slices undergoing frying and to translate these relationships into appropriate equations that can be used to calculate the time required to reach a given moisture content.

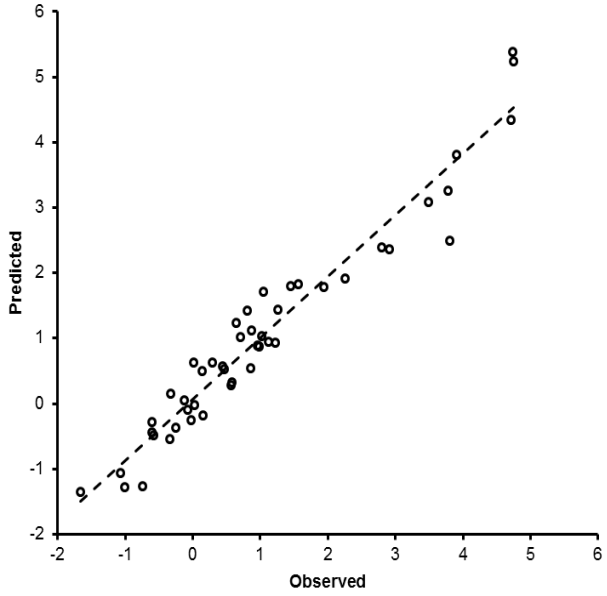


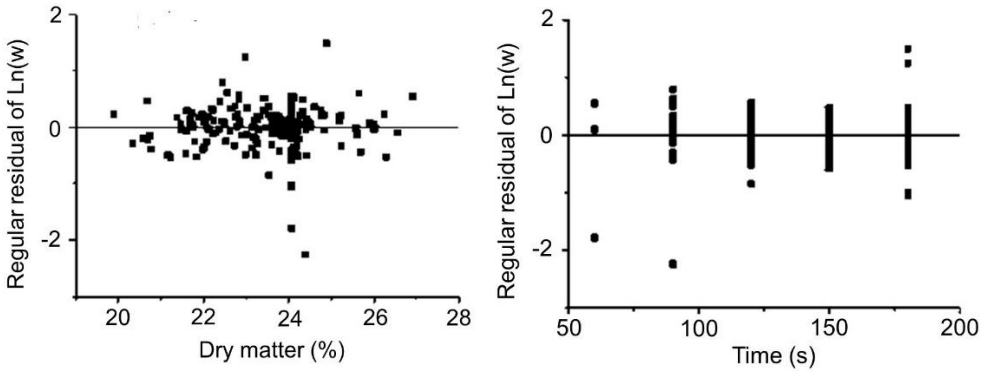
Figure 1. Predicted *vs* observed values of LnW when dehydration-time data were fitted to the following quadratic model: $LnW = 20.99 – 0.07t + 0.14T – 1.98M + 2.9x10^{–4}Tt – 2.1x10^{–3}Mt – 0.01MT + 1.9x10^{–4}t^2 + 0.09M^2$ where W is the moisture content (% dwb) at frying time t (s), T is the temperature of the frying oil in °C and M is the dry matter content (%) of the potatoes used for the dehydration experiments (T=150-190 °C, t=54-200 s) (*n*=50).

Measured moisture-content data were shown to conform to a quadratic model, whose equation was rearranged into a quadratic in t as follows: $At^2 + (BT–CM–D)t + (E+FT–GM–HMT+IM2–LnW) = 0$ which could be solved for time at any value of LnW (**Figure 1**). T and M have the same significance as stated in Figure 1 and A- I are parameters corresponding to those in the original quadratic model. The values of these parameters were obtained by fitting the quadratic in t to the whole moisture-temperature-dry matter-time dataset obtained (selecting moisture contents <6% dwb) in the kinetic experiments for the formation of acrylamide detailed in this study with results given in **Table 1** and the corresponding

residuals plots shown in **Figure 2**. Despite the standard errors associated to correlation effects, the quadratic in t provides a reliable method to determine the time at which specific moisture-contents may be achieved given the oil temperature and dry matter content of the original potato sample. The plots of residuals for the estimated parameters indicate that the three variables (t, M and T) are accounted for adequately by the model.

Table 1. Parameters A–I for the quadratic in t obtained from moisture-temperature-dry matter-time iterations (n=50)

Parameter	Value	Standard error	Units
A	1.02×10^{-4}	0.28×10^{-4}	s^{-2}
B	0.42×10^{-4}	1.13×10^{-4}	$(°C)^{-1}s^{-1}$
C	-1.05×10^{-3}	0.79×10^{-3}	s^{-1}
D	0.0695	0.0285	s^{-1}
E	19.4	15.8	none
F	-0.0389	0.0943	$(°C)^{-1}$
G	0.533	0.826	none
H	0.65×10^{-3}	3.81×10^{-3}	$(°C)^{-1}$
I	0.0103	0.015	none



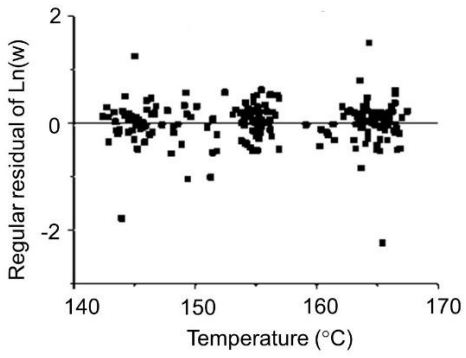


Figure 2. Residuals plots for the determination of parameters given in Table 1. Dry matter measured in triplicate and temperature in 12 replicates per treatment.

Kinetic modelling - general considerations. The purpose of this kinetic modelling is to provide a mathematical description of the time-dependent formation of acrylamide during the frying operation such that the model can subsequently be used to describe acrylamide outcomes. The variables to be taken into account are potato composition (sugars, amino acids), frying temperature, and final moisture content. Of particular importance is the need to ensure that the resulting kinetic model is the simplest robust formulation of the kinetics; the starting point was the stepwise process (Parker et al., 2012) validated for the frying of potato fries illustrated in **Figure 3**.

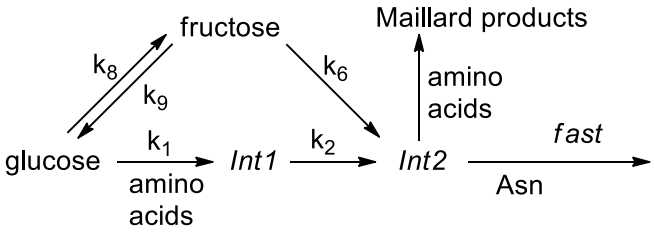


Figure 3. Simplified kinetic model for the formation of acrylamide from a reducing sugar and asparagine (Parker et al., 2012).

With no significant interconversion between glucose and fructose on the timescale of the frying process (Parker et al., 2012), the key assumptions of the model are that there is a common intermediate (Int2) in the formation of Maillard products and acrylamide, and the resulting yield of acrylamide is determined amongst others, by competition between asparagine and the pool of amino acids. This intermediate may be formed both by the Maillard reaction and by the 'caramelization' of fructose. The kinetics of acrylamide formation were studied in samples subjected to the five glucose and five fructose treatments. At 155 °C, the formation of acrylamide initiated after 80-95 s of frying (**Figure 4a**), at which point the moisture content of the crisps was ≤6%, the formation then proceeded at a constant rate over the observation period. At fixed amino acid composition ([TAA]=68.0 mmol/kg) the resulting rate of acrylamide formation was found to be proportional to the initial concentration of both glucose and fructose (**Figure 4b**) suggesting that the reaction is of first order with respect to both sugars. The first order rate constants were calculated as 1.6 and 3.2 ppb kg mol^{-1} s^{-1} for the reactions of glucose and

fructose, respectively, suggesting that, in this system, fructose was twice as reactive as glucose with regard to the formation of acrylamide.

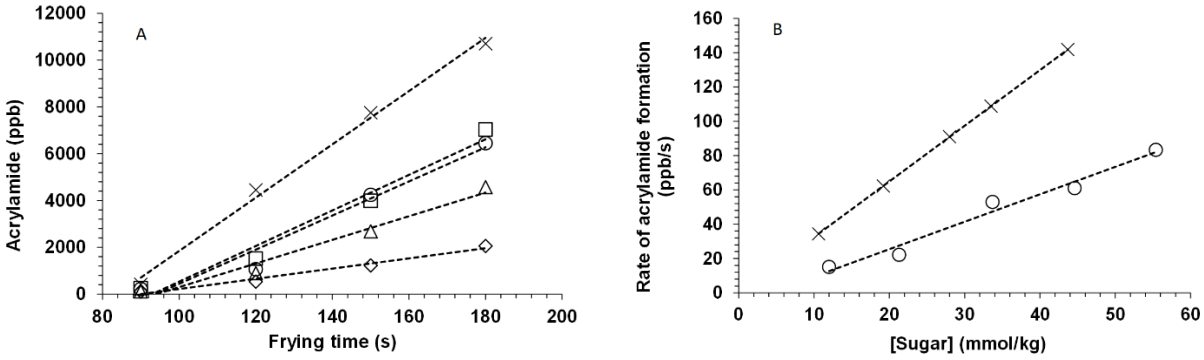


Figure 4. **A**) Typical acrylamide concentration-frying time plot illustrated here for initial [fructose] = X 141.9 mmol/kg; ○ 108.8 mmol/kg; □ 91.0 mmol/kg; △ 62.3 mmol/kg; ◇ 34.4 mmol/kg in pooled samples (*n*=27, single measurement) at 155 °C. **B**) Dependence of rate of formation of acrylamide on initial [glucose] (○) and [fructose] (×) at 155 °C obtained from five 6-point kinetic runs for each reducing sugar.

Previous studies (Amrein et al., 2003) had similarly found that fructose was approximately twice as important as glucose in acrylamide formation in hash brown systems while fructose was significantly more important than glucose in the formation of acrylamide in model systems (Pollien, Lindinger, Yeretzian, & Blank, 2003). However, no significant difference in acrylamide formation between model systems containing fructose, galactose, lactose and glucose were found (Stadler et al., 2002), while in yeast-leavened wheat bread increased fructose did not significantly impact the formation of acrylamide (Surdyk, Rosen, Andersson, & Aman, 2004). Furthermore, Parker et al. observed that glucose concentration decreased twice as quickly as fructose concentration during the finish-frying of French Fries but postulated that their relative reactivities would change when potato cultivars with lower amino acid contents are studied. The corresponding moisture content at the end of the initial lag stage (80-95 s) before acrylamide formation commences at 155 °C and when the potato slices undergo dehydration (concentration of the reactants increasing) was determined experimentally to be approximately 6% (dwb), which effectively coincides with the system reaching its isothermal state, thereby obviating any need for complex heat and mass transport calculations during the effective

reaction period. The average moisture content at 155 °C (90 s) was 5.91 (n=12, sd 1.75) while similar results were obtained at 165 °C (n=22) and at 145 °C but at longer frying times (120 s, n=22). Although, the proposed kinetic model can only be phenomenological since the heat and mass transport behaviour during the early stages of frying is not yet understood, on the basis of the model shown in **Figure 3** and the kinetic data, the simplest rate equation for the formation of acrylamide is suggested as follows:

$$\frac{d[acryl]}{dt} = k_1[glu][TAA]\frac{[asn]}{[TAA]} + k_6[fru]\frac{[asn]}{[TAA]} \qquad (1)$$

Where [TAA] is the concentration of total amino acids. Thus, in the initial rate phase, the concentration of acrylamide formed after a time t is given by:

$$[acryl]_t = k_1[glu][asn]t + k_6[fru]\frac{[asn]}{[TAA]}t \qquad (2)$$

The critical variable is the reaction time t. The hypothesis to be tested here is that the formation of acrylamide begins at a 'critical' value of moisture content and that the effective reaction time may be calculated from the rate of dehydration at a given frying temperature and the final moisture content to be achieved.

Amino acid concentrations are grouped together as TAA. The validity of this approach was tested by including in the experimental protocol fortification of the potato slices with glutamine and glycine to determine any specific kinetic effects from these two amino acids with widely differing structures.

Model implementation. The initial concentrations of the major reactants were varied independently ([glucose]=32–217 mmol/kg, [fructose]=35–159 mmol/kg, [asn]=135–328 mmol/kg, [gln]=7–77 mmol/kg, [gly]= 0.1 mmol/kg and 48–164 mmol/kg, with [TAA]= 35–350 mmol/kg and [asn]/[TAA]=0.07–0.88) and the concentration of acrylamide was measured at 90, 120, 150 and 180 s frying time at oil temperatures nominally at 145, 155 and 165 °C. The moisture- and fat-contents of the fried product were determined and acrylamide concentrations and moisture levels were expressed on a dry weight non-fat basis. The reaction time was calculated by setting the critical moisture content to 6% (dwb) and calculating the time for the samples to reach the critical and final moisture contents using the 'quadratic formula' (with negative square root) to solve the quadratic in t. The reaction time was taken as the difference between the two calculated results. In the final calculation of the model, only data

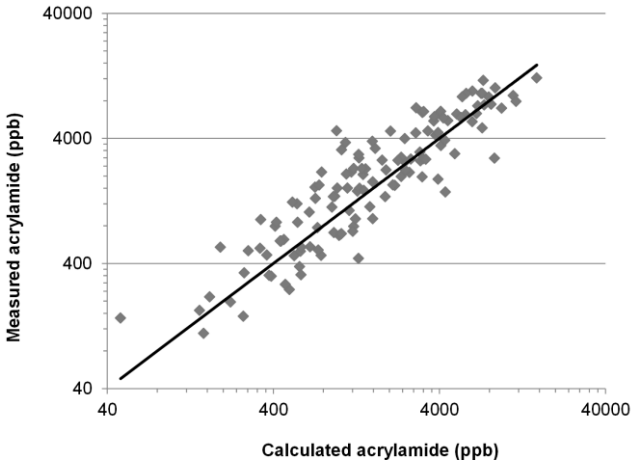
corresponding to a measurable rate of acrylamide formation, i.e., when the moisture content of the fried product was <c. 6% dry weight non-fat basis, were included.

The model was coded in Excel according to **Equation 2** with the addition of the temperature dependence of k_1 and k_6 according to the Arrhenius equation (**Equation 3**).

$$k_1 = A_1\, e^{-E_a/RT} \quad \text{and} \quad k_6 = A_6 e^{-E_a/RT} \qquad (3)$$

where A_1 and A_6 are the corresponding pre-exponential terms, E_a is the activation energy, R=8.314 J mol^{-1} K^{-1} and T is the absolute temperature.

The fit of the model to the experimental data is illustrated in **Figure 5** which spans a hundred-fold range in acrylamide yields. The model explains well the variation in acrylamide due to the experimental variables as illustrated by the plots of residuals.



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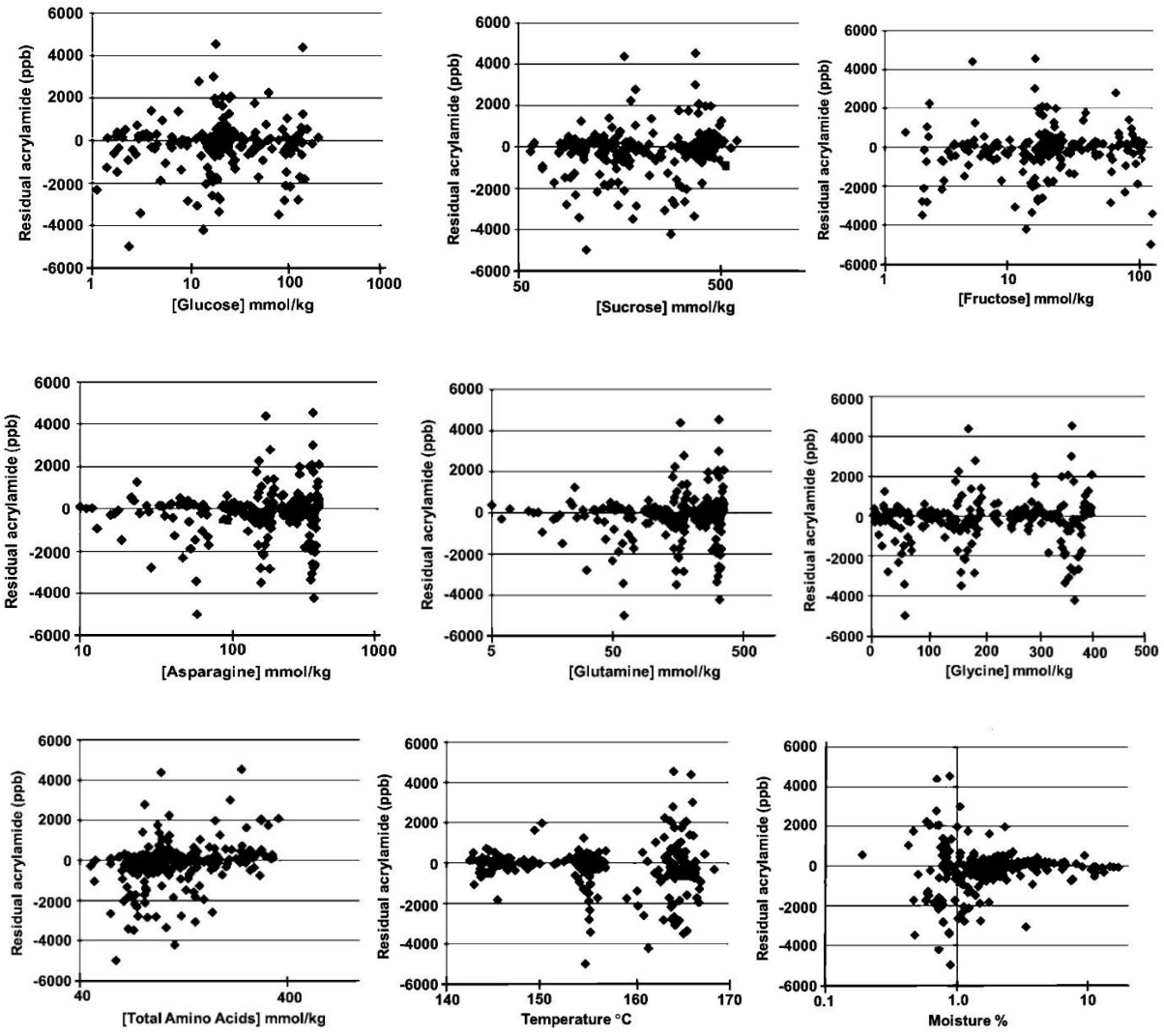

315 **Figure 5**. Fit of the kinetic model and residual plots given by equation 2 to kinetic data over a wide
316 concentration range of sugars and amino acids and at three frying temperatures (145, 155, 165 °C)
317 (*n*=214).

318 The kinetic parameters calculated for the data illustrated in **Figure 5** were A_1=1.29x10^{12} and
319 A_6=1.19x10^{10}, the pre-exponential terms corresponding to k_1 and k_6 (**equation 3**) and W_0 (6%) the
320 critical moisture content for the reaction to commence. Whereas the activation energy E_a (95 kJ/mol) is
321 assumed to be the same for the reactions of glucose and fructose, the model is unable to resolve the
322 contributions from the individual reactions.

323 The model depicted by **equation 1** is somewhat simpler than that proposed previously (Parker et al.,
324 2012) for the finish-frying of French fries, the latter comprising a number of consecutive rate-limiting

steps whereas **equation 1** is consistent with the occurrence of two parallel, single-step reactions. Whether or not the chemical mechanism for the formation of acrylamide is the same in French fries and PCs, dehydration processes and internal temperature profiles during the frying of PCs and French fries are very different hence, it is not surprising that the kinetics of acrylamide formation in the two processes differ.

CONCLUSIONS

The pre-treatment of raw potato slices using a combined blanching and fortification process successfully altered the chemical composition of the slices, providing a range of compositions suitable for kinetic studies. The relationship between the amount of solute dissolved in the blanching liquor and the uptake observed in the potato slices, after the 1 min exposure to the broth at 78 °C, was linear.

The rate of dehydration of PC with differing dry matter contents was investigated at specific intervals determined through the factorial experimental design. The data were used to formulate a quadratic equation for the time at which potato slices reached given moisture contents during frying and to calculate the effective reaction time, there being a critical moisture content of 6% (dwb) at which acrylamide formation began.

The chemical kinetic model was found to conform to the following rate equation:

$$\frac{d[acryl]}{dt} = k_1[glucose][asn] + k_6[fructose]\frac{[asn]}{[TAA]}$$

Where TAA represents the total amino acid concentration. The timescale of the frying process meant that the chemical reactions were all in their initial rate phase. The adoption of initial rates implies that initial concentrations were maintained throughout the observation period and is part of the overall hypothesis which is validated through modelling.

As a study in chemical kinetics, the range of reactant concentrations and ratios of [asn]/[TAA] were sufficiently large to elicit the specific behaviors of glucose and fructose, and of asparagine relative to the total amino acid pool. Thus, kinetic parameters confirm that the fructose-dependent reaction (caramelization) contributes twice as much acrylamide as the reaction of glucose (Maillard reaction).

Similarly, the fact that the outcomes of kinetic runs with modified concentrations of asparagine, glutamine and glycine can be predicted using the corresponding grouped concentration of amino acids implies that, within the accuracy of the available kinetic data, it is perfectly in order to treat the total amino acid concentration as a variable despite the species being made up of 20 or more amino compounds. We believe that this investigation is the most rigorous and detailed study of the kinetics of acrylamide formation in any food process and potentially provides the foundation for further optimizing acrylamide mitigation in PC according to the ALARA principle based on sugar and amino acid composition of potatoes.

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Kinetic Modelling of Acrylamide Formation during the Frying of Potato Chips

Matthew Knight[2], Simon McWilliam[1], Sarah Peck[1], Georgios Koutsidis[2*], Gemma Chope[1], Ian Puddephat[1]and Bronislaw Wedzicha[3]

[1] PepsiCo International Ltd, Beaumont Park, 4 Leycroft Road, Leicestershire, LE4 1ET, UK

[2] Department of Applied Sciences, University of Northumbria at Newcastle, Newcastle City Campus, Ellison Place, Newcastle upon Tyne, Tyne and Wear, NE1 8ST

[3] School of Food Science and Nutrition, University of Leeds, Leeds LS2 9JT

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*Corresponding author at: Department of Applied Sciences, University of Northumbria at Newcastle, Newcastle City Campus, Ellison Place, Newcastle upon Tyne, Tyne and Wear, NE1 8ST, UK.

Tel.: +44 191 227 3978, E-mail address: Georgios.koutsidis@northumbria.ac.uk

ABSTRACT: The effect of potato tuber composition, frying time and temperature on acrylamide formation in potato chips was investigated and a mathematical model of the kinetics of acrylamide formation is provided. Moisture-temperature-time profiles were obtained for potato slices during frying to enable the determination of the 'effective' reaction time by identifying the critical moisture content (6% dwb) for acrylamide formation to commence and using dehydration curves to calculate subsequent frying time to finished product moisture-content. The chemical kinetic model conformed to the following rate equation over a one hundred-fold range of acrylamide concentrations:

$$\frac{d[acryl]}{dt} = k_1[glucose][asn] + k_6[fructose]\frac{[asn]}{[TAA]}$$

where [TAA] represents total amino acid concentration. The timescale of the frying process meant that the chemical reactions were all in their initial rate phase. Kinetic parameters confirm that the fructose-dependent reaction (caramelization) contributes twice as much acrylamide as the reaction of glucose (Maillard reaction).

INTRODUCTION

Acrylamide is a very well-known process contaminant formed *via* the Maillard reaction between reducing sugars and/or carbonyl compounds and the amino acid asparagine (Mottram, Wedzicha, & Dodson, 2002) in several different foodstuffs including potato-based, cereal-based foods and coffee (Lineback, Coughlin, & Stadler, 2012). In potato-based products such as potato chips and French fries, the combination of the thermally intense frying process and the high native levels of asparagine may generate significant levels of acrylamide, 117–4,215 $\mu g\ kg^{-1}$ and 59–5,200 $\mu g\ kg^{-1}$ respectively (Lineback et al., 2012). Since the discovery of acrylamide in foods in 2002, food businesses and researchers worldwide have undertaken substantial research activities resulting in over 1,400 publications, investigating formation mechanisms, kinetics, occurrence and exposure, toxicology and mitigation strategies. Information were compiled into national databases, the FoodDrinkEurope acrylamide toolbox (currently in its 15th edition) and scientific opinion documents (EFSA CONTAM panel, 2015) leading to Commission Regulation (EU) 2017/2158 establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in foods. Amongst others, where possible, control and mitigation strategies proposed were the selection of raw materials based on asparagine and reducing sugar levels, re-evaluation of agronomy practices, processing and/or storage conditions of raw materials and reformulation strategies incorporating acrylamide mitigation agents. Mathematical models predicting acrylamide yields have also been proposed as a means to control and monitor acrylamide content in end products and a number of approaches have been followed including pattern recognition (Pedreschi, Segtnan, & Knutsen, 2010), artificial neural networks (Serpen & Gökmen, 2007) and kinetics (Parker, Balagiannis, Higley, Smith, Wedzicha, & Mottram, 2012). Such approaches are of significant value to the industry as they could provide predicting and monitoring capabilities in addition to the relatively laborious analytical determinations of acrylamide content. Moreover, associations between important quality parameters such as colour, end moisture and acrylamide could also be made, thus providing a useful quality control tool that links physicochemical properties of end-products with consumer safety. However, most pattern recognition and neural network approaches as well as simple colour-acrylamide or moisture-acrylamide kinetic models may require

frequent re-validation exercises in cases of changes in raw materials and/or process adjustment. The present study recognises the opportunity for a quantitative tool for potato crisp (PC) production using material composition, process conditions (temperature, time) and finished product parameters (*e.g.* moisture content) to predict acrylamide levels while allowing for variation in production practices. The hypothesis being tested is that a combination of systematic adjustment of potato slice-composition to achieve a wide a range of sugar and amino acid ratios coupled to precise control of process conditions in a bench top PC fryer will allow for the development of a robust yet simplified kinetic modelling of acrylamide formation specific to PC processing.

METHODS AND MATERIALS

Materials. Fresh potato tubers (cv. Lady Rosetta) used in the kinetic studies were sourced from two stocks from Leycroft Road Walkers Factory, Leicester, UK. Tubers (cv. Lady Rosetta) used for the determination of dehydration rates were sourced from two cultivation locations in the UK, specifically North Yorkshire and Norfolk, to provide a range of Dry Matter (DM) contents.

Chemicals. Glucose, fructose and sucrose were all sourced from Sigma-Aldrich Co., Ltd. (Poole, Dorset, U.K.) and were of >99% purity. Asparagine, glutamine and glycine were sourced from Sigma-Aldrich Co., Ltd. (Poole, Dorset, U.K.) and Fischer Scientific (Loughborough, Leicestershire, U.K.) and were of >97% purity.

Preparation of sugar- and amino acid- fortified potato slices for kinetic studies. Tubers were graded for size (producing PC with a diameter of 50-70 mm) and sliced using an industrial slicer to a standard PC thickness with a target coefficient of variation (CV) of <11%. The stock potato broth was made by adding 2.5 kg of fresh, washed and peeled potato slices to a freestanding heated water tank containing 25 L of water at 80 °C. The slices were placed inside a muslin sack and submerged in water for 1 min under continuous agitation to ensure equal and reproducible mass transfer (inter- and intra-cellular component leaching). The slices were then removed and discarded and the broth contained within the tank was kept at 80 °C. Fortification of sugars through blanching involved treatments with12 sugar solutions, namely; 0.2, 0.4, 0.6, 0.8 and 1.0% w/v glucose, 0.2, 0.4, 0.6, 0.8 and 1.0% w/v fructose and

68 0.2%/0.2% w/v glucose/fructose and finally 0.2%/0.2%/0.2% w/v glucose/fructose/sucrose.
69 Fortification of amino acids through blanching involved treatments with 10 amino acid solutions,
70 namely; 1.5, 2.0, 2.5, 3.0 and 3.5% w/v asparagine, 0.6% and 1.2% w/v glutamine and 0.5, 1.0 and
71 2.0% w/v glycine.

72 Sugar- or amino acid- fortified blanching broths (1.25 kg) were prepared in a metal bowl and transferred
73 to a water bath (Nickel Electro Ltd, Weston-super-Mare, UK) set at 80 °C. The temperature of the
74 fortified broths was measured using a handheld digital thermometer (RS Components Ltd., Northants,
75 UK). Upon reaching 78 °C, 50 potato slices were placed in the metal bowl and stirred continuously for
76 1 min. The blanched slices were transferred from the solution onto paper towels to remove the excess
77 liquid. Eighteen slices were taken, snap frozen in a liquid nitrogen cradle for 90 s, ground (for <15 s)
78 to a fine powder using a WSG 30K Spice Grinder (Conair Corp., Stamford, CT), placed in 150 mL
79 plastic sample pots and transferred to a -80 °C freezer until compositional determinations.

80 Blanched slices (*n*=27) were placed individually into a custom-built 27 cell fry basket. The fry basket
81 was reassembled and submerged into the deep fat fryer (Bartlett Yeoman, Yeoman) for pre-defined times
82 (30-180 s) and oil temperatures (145-165 °C) according to the experimental design. Temperature data
83 were recorded in each corner of the frying basket using a datalogger (Thermosense, Manchester, UK).
84 After frying, the PCs were removed from the oil and allowed to cool for 4-5 min before being placed
85 into a -20 °C freezer for storage, pending analysis. The approach resulted in 480 kinetic runs across 4
86 temperatures and 80 potato slice compositions.

87 **Determination of dehydration curves during PC frying.** The effect of the dry matter-content (DM)
88 on dehydration rates during frying was examined using a factorial experimental design of target
89 temperature (150-190 °C), across three categories of DM (21, 23 and 25%, (sd = ± 0.33)) and time (54-
90 200 s), comprising of 50 experimental conditions (*n*=27 slices for each condition) using Design Expert
91 software. Two batches of Lady Rosetta potatoes were sorted and graded to include tubers in the 50-
92 70 mm range from rose to heel. Each tuber was weighed, both in air and in water and the dry-matter
93 content for each potato was calculated. Tubers for each category of DM were rumble-peeled and washed
94 before being sliced to the standard PC thickness. The slices were rinsed briefly in cold water, surface

95 water was removed by patting them dry with a paper towel and subsequently fried as determined by the
96 experimental design. The procedure for frying, cooling and storage was identical to that detailed above.

97 **Analysis of sugars by Ion Chromatography (IC).** Aliquots of snap-frozen, homogenised potatoes
98 (8.5±0.1 g) were weighed into 50 mL centrifuge tubes, 20 mL of UHP Water (18.2 Ω) were added and
99 the samples were mixed thoroughly by inversion. The tubes were subsequently vortexed for 1 min, the
100 contents left to settle for 5 min and centrifuged at 4500 rpm for 2 min at 8 °C. Ethanol (3 mL, 96%)
101 was added to 15 mL centrifuge tubes followed by 1 mL of the supernatant and the samples were mixed.
102 The addition of sample to the ethanol took place within 15 min of the sample being weighed into the
103 50 mL centrifuge tube. Extracts were diluted (1:50 (v/v)) in UHP water and aliquots (20 µL) analysed
104 using a Dionex ICS 3000 system equipped with a CarboPac PA20 analytical column (3 mm x 150 mm)
105 and a CarboPac PA20 guard column (3 mm x 30 mm). The mobile phase was generated using an Elugen
106 III KOH cartridge. The eluent concentration was 15 mM at a flow rate of 0.37 mL/min and the oven
107 and detector temperature were set at 25 °C. A gold working electrode and pH Ag/AgCl combination
108 reference electrode were used in the carbohydrate setting EC waveform. The total run time of the
109 method was 22 min.

110 **Analysis of amino acids by GC-MS (EZ:Faast Method).** The EZ:Faast GC-MS kit was used for free
111 amino acid analysis as described previously (Elmore, Mottram, Muttucumaru, Dodson, Parry, &
112 Halford, 2007). Samples were analysed using an Agilent 5975 GC-MS equipped with a ZB-AAA
113 capillary column (10 m x 250 µm x 0.25 µm). Samples (2 µL) were injected at 250 °C in split mode
114 (10:1) using helium as a carrier gas at a flow rate of 1.1 mL/min. The initial oven temperature was set
115 at 110 °C and immediately followed by a temperature ramp to 320 °C at 30 °C /min. The transfer line,
116 ion source and quadrupole temperatures were set at 280 °C, 240 °C and 180 °C, respectively. The mass
117 spectrometer was operated in the total ion scan mode (*m/z* 45-450) and *m/z* 155 and 160 were used as
118 the quantitation ions for asparagine and ^{13}C-asparagine, respectively.

119 **Loss on drying.** Aliquots of snap-frozen, homogenised potato (2-3 g) were weighed into pre-dried,
120 cooled in a desiccator and accurately weighed stainless steel dishes. The samples were then placed in a

121 forced-air oven at 100±2 °C for 2 days, cooled in a desiccator, weighed and the % loss on drying
122 calculated.

123 **Determination of moisture-content.** Moisture-content was determined gravimetrically on a pooled
124 subsample of the fried product obtained according to the factorial experimental designs described
125 previously. Samples were crushed, weighed accurately (1-2 g, 4 d.p.), placed in an industrial oven at
126 105 °C for 18 hours, cooled in a desiccator and reweighed.

127 **Analysis of acrylamide by LC-MS/MS.** Fried PC samples (25 g) were homogenised in a Robot Coupe
128 food processor using 5 pulses. Homogenised samples (1.00±0.02 g) were weighed into a 12 mL
129 centrifuge tube, internal standard solution (10 mL of 20 ppb $^{13}C_3$ acrylamide in HPLC grade methanol)
130 was added and followed by vortex mixing. Samples were then placed on a shaker mixer for 20 min and
131 subsequently centrifuged at 13500 rpm for 10 min at 25 °C. Automated purification of the crude extracts
132 was performed by transferring aliquots (300 µL) to a primed 96 well Bond Elut plate (Agilent
133 Technologies Inc., Santa Clara, United States) using a Hamilton STARlet robot (Hamilton Bonaduz
134 AG, Bonaduz Switzerland) coupled to a Cerex 96 multichannel SPE Positive Pressure Processor
135 (SPEware Corp, Baldwin Park, California, United States). Low pressure was applied to the plate 30 min
136 after sample addition and for 5-10 s. UHP water (300 µL) was added into each sample well and left to
137 stand for 15 min followed by a second low pressure application step for 5-10 s. Both the initial sample
138 elution and wash were discarded and a fresh plate was used for the elution and collection of the purified
139 extract using 150 µL of UHP water, followed by standing for 15 min and application of low pressure to
140 the plate for 5-10 s until the plate filters were dry.

141 LC-MS/MS analyses were performed using an Applied Biosystems API 5000 LC/MS/MS system
142 equipped with an Agilent 1200 HPLC pump and a Gerstel Multipurpose Sampler 2XL. Ten microliters
143 of sample were injected on a Hypercarb column (100 mm x 3 mm, 5 µm, Thermo Scientific, Waltham,
144 USA) thermostated at 60 °C. The mobile phase consisted of deionised water: methanol: formic acid
145 850:150:1.0 (v/v/v) with a flow rate of 0.25 mL/min. The following transitions were used for
146 quantitation purposes: quantification ion 72→55, (declustering potential 55 V, collision energy 25 eV,
147 collision cell exit potential 10 V), confirmation ion 72→44, (declustering potential 51.3 V, collision

148 energy 47 eV, collision cell exit potential 18.4 V), internal standard ion 75→58, (declustering potential
149 55 V, collision energy 25 eV, collision cell exit potential 10 V).

150 **Determination of extractable oil using the Soxhlet method.** Extractable oil of the PC samples was
151 determined using the Campden BRI method - TES-AC-536, which encompasses a Weibull-Stoldt
152 extraction (acid hydrolysis followed by Soxtec extraction) and crude fat (Soxtec) extraction.

153 **RESULTS AND DISCUSSION**

154 **Infusion Blanching Fortification of Potato Slices.** The objective was an experimental system which
155 allowed for the tuber sugar and amino acid composition to be varied independently without the
156 constraints of native composition. Glucose (0.2, 0.4, 0.6, 0.8, 1.0% w/v), fructose (0.2, 0.4, 0.6, 0.8,
157 1.0% w/v) and sucrose (0.2% w/v) fortified broths allowed for the study of their individual roles in
158 acrylamide formation. Similarly, asparagine, glutamine and glycine broths allowed for the study of
159 reactions related to both acrylamide formation (asparagine) as well as competing Maillard reaction
160 pathways (glutamine and glycine). Additionally, fortification of the potato samples with different
161 amounts of amino acids relative to asparagine allowed the examination of the effect of the ratio of
162 asparagine: total amino acid which has been shown to correlate to acrylamide formation in potato-based
163 systems, under low sugar conditions (Elmore et al., 2007). Linear relationships between the
164 concentration of the fortifying solute(s) in the blanching broth and their concentration in the blanched
165 slice were observed on each occasion.

166 During blanching of potato slices, the high temperatures disrupt the cell membranes of the potato,
167 allowing components both from within the cell and in the broth to diffuse across the gradient (Arroqui,
168 Rumsey, Lopez, & Virseda, 2002; Bartlett, et al., 2020). To prevent excessive leaching of the native
169 components and, in turn, shift the mass transfer equilibrium, the blanching process utilised a 'broth'
170 made from blanched potato slices in the same potato: water ratio (1:10 w/v).

171 The fortification of slices with asparagine and glycine was conducted to achieve a substantial difference
172 in asparagine: total amino acid (Asn:TAA) ratios across the experimental design. The Asn:TAA ratios
173 ranged from 0.11 to 0.79; these limits were achieved by infusing in a 2.0% glycine and 3.5% asparagine

174 solution, respectively. The average ratio in blanched control samples was 0.36. A total of 480 kinetic
175 runs were obtained across 4 temperatures and 80 composition treatments. The infusion process resulted
176 in an average RSD of 20% for sugars and 24% for amino acids.

177 Previous studies had been concerned with correlation between the ratios of asparagine to total amino
178 acids and acrylamide formation in potato products (Becalski et al., 2004; Elmore et al., 2007). Elmore
179 et al. showed that when the sugar concentration of potato varieties is low, the level of asparagine relative
180 to the total amino acid content of the tuber becomes a driving factor in acrylamide formation. Low sugar
181 levels limit the formation of acrylamide and it is at such low levels that competition of asparagine with
182 other amino acids becomes most important. Becalski et al., on the other hand, did not find such
183 correlations when examining the three-dimensional relationship between Asn:TAA ratio, sugar
184 concentration and acrylamide yield; however, the authors utilised a range of 0.12-0.38 Asn:TAA, which
185 may be too narrow to identify a global trend that is also valid at high asparagine contents.

186 **PC dehydration rates.** Commercially produced PC have an average moisture content less than 2.0%
187 (Kita, Bråthen, Knutsen, & Wicklund, 2004; Wu, Jouhara, Tassou, & Karayiannis, 2012). Moisture
188 plays a critical role in acrylamide formation, with low moisture-high temperature conditions (roasting,
189 frying, baking) generating higher acrylamide yields (Wicklund, Ostlie, Lothe, & Kita, 2006). The
190 thermal input, specifically the heating time and temperature, is key for PC dehydration and the
191 formation of acrylamide (FoodDrinkEurope, 2019). The water loss associated with high temperature
192 treatments occurs primarily in the outer layer of foodstuff, such as the outer layer of French fries or
193 bread. It is suggested here that, owing to the potato slices being relatively thin, dehydration of PCs
194 occurs at an approximately constant rate throughout the slice during frying and, thus, acrylamide
195 formation is also deemed to be uniform across the entire slice.

196 The greatest uncertainty in the modelling of the kinetics of the frying process is the actual temperature
197 at which the reactions take place and the reaction time. The hypothesis being tested here is that the
198 formation of acrylamide begins when the moisture content of the PC slice falls to a 'critical' value and
199 since, at that time the slice would have lost a high proportion of its moisture, the reaction will occur
200 isothermally at, effectively, the final temperature of the frying oil. The reaction time would then be the

time from the start of the reaction to that required to achieve the final moisture content. The experimental plan was, therefore, to plot moisture-temperature-time relationships for potato slices undergoing frying and to translate these relationships into appropriate equations that can be used to calculate the time required to reach a given moisture content.

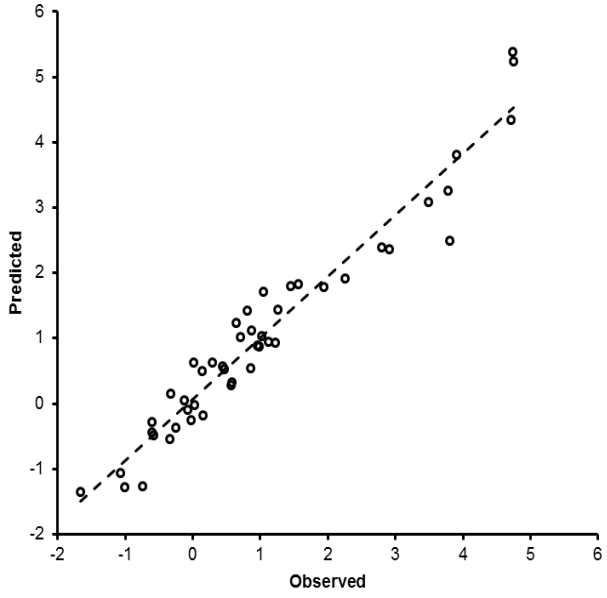


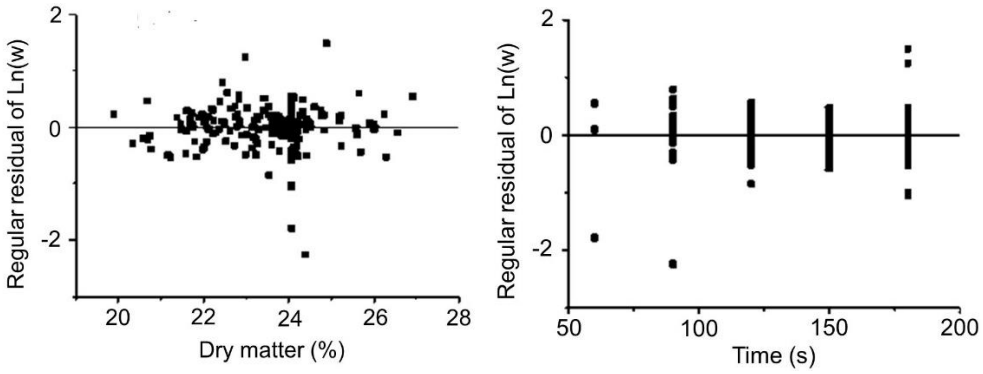
Figure 1. Predicted *vs* observed values of LnW when dehydration-time data were fitted to the following quadratic model: $LnW = 20.99 - 0.07t + 0.14T - 1.98M + 2.9x10^{-4}Tt - 2.1x10^{-3}Mt - 0.01MT + 1.9x10^{-4}t^2 + 0.09M^2$ where W is the moisture content (% dwb) at frying time t (s), T is the temperature of the frying oil in °C and M is the dry matter content (%) of the potatoes used for the dehydration experiments (T=150-190 °C, t=54-200 s) (*n*=50).

Measured moisture-content data were shown to conform to a quadratic model, whose equation was rearranged into a quadratic in t as follows: $At^2 + (BT–CM–D)t + (E+FT–GM–HMT+IM2–LnW) = 0$ which could be solved for time at any value of LnW (**Figure 1**). T and M have the same significance as stated in Figure 1 and A- I are parameters corresponding to those in the original quadratic model. The values of these parameters were obtained by fitting the quadratic in t to the whole moisture-temperature-dry matter-time dataset obtained (selecting moisture contents <6% dwb) in the kinetic experiments for the formation of acrylamide detailed in this study with results given in **Table 1** and the corresponding

residuals plots shown in **Figure 2**. Despite the standard errors associated to correlation effects, the quadratic in t provides a reliable method to determine the time at which specific moisture-contents may be achieved given the oil temperature and dry matter content of the original potato sample. The plots of residuals for the estimated parameters indicate that the three variables (t, M and T) are accounted for adequately by the model.

Table 1. Parameters A–I for the quadratic in t obtained from moisture-temperature-dry matter-time iterations (n=50)

Parameter	Value	Standard error	Units
A	1.02×10^{-4}	0.28×10^{-4}	s^{-2}
B	0.42×10^{-4}	1.13×10^{-4}	$(°C)^{-1}s^{-1}$
C	-1.05×10^{-3}	0.79×10^{-3}	s^{-1}
D	0.0695	0.0285	s^{-1}
E	19.4	15.8	none
F	-0.0389	0.0943	$(°C)^{-1}$
G	0.533	0.826	none
H	0.65×10^{-3}	3.81×10^{-3}	$(°C)^{-1}$
I	0.0103	0.015	none



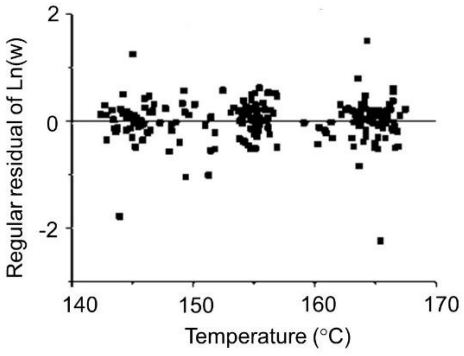


Figure 2. Residuals plots for the determination of parameters given in Table 1. Dry matter measured in triplicate and temperature in 12 replicates per treatment.

Kinetic modelling - general considerations. The purpose of this kinetic modelling is to provide a mathematical description of the time-dependent formation of acrylamide during the frying operation such that the model can subsequently be used to describe acrylamide outcomes. The variables to be taken into account are potato composition (sugars, amino acids), frying temperature, and final moisture content. Of particular importance is the need to ensure that the resulting kinetic model is the simplest robust formulation of the kinetics; the starting point was the stepwise process (Parker et al., 2012) validated for the frying of potato fries illustrated in **Figure 4**.

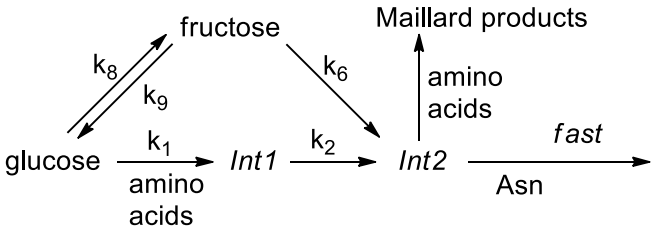
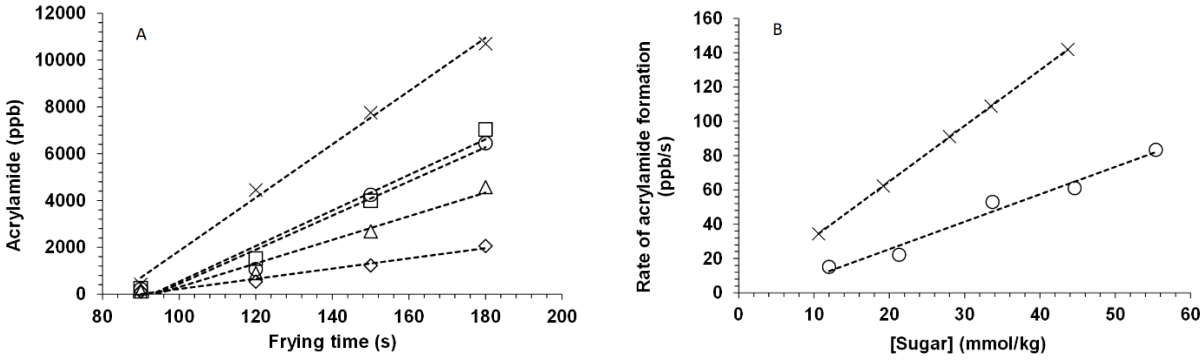


Figure 3. Simplified kinetic model for the formation of acrylamide from a reducing sugar and asparagine (Parker et al., 2012).

With no significant interconversion between glucose and fructose on the timescale of the frying process (Parker et al., 2012), the key assumptions of the model are that there is a common intermediate (Int2) in the formation of Maillard products and acrylamide, and the resulting yield of acrylamide is determined amongst others, by competition between asparagine and the pool of amino acids. This intermediate may be formed both by the Maillard reaction and by the 'caramelization' of fructose. The kinetics of acrylamide formation were studied in samples subjected to the five glucose and five fructose treatments. At 155 °C, the formation of acrylamide initiated after 80-95 s of frying (**Figure 4a**), at which point the moisture content of the crisps was ≤6%, the formation then proceeded at a constant rate over the observation period. At fixed amino acid composition ([TAA]=68.0 mmol/kg) the resulting rate of acrylamide formation was found to be proportional to the initial concentration of both glucose and fructose (**Figure 4b**) suggesting that the reaction is of first order with respect to both sugars. The first order rate constants were calculated as 1.6 and 3.2 ppb kg mol^{-1} s^{-1} for the reactions of glucose and

253 fructose, respectively, suggesting that, in this system, fructose was twice as reactive as glucose with
254 regard to the formation of acrylamide.



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256 **Figure 4. A**) Typical acrylamide concentration-frying time plot illustrated here for initial [fructose] =
257 X 141.9 mmol/kg; ○ 108.8 mmol/kg; □ 91.0 mmol/kg; △ 62.3 mmol/kg; ◇ 34.4 mmol/kg in pooled
258 samples (*n*=27, single measurement) at 155 °C. **B**) Dependence of rate of formation of acrylamide on
259 initial [glucose] (○) and [fructose] (×) at 155 °C obtained from five 6-point kinetic runs for each
260 reducing sugar.

261 Previous studies (Amrein et al., 2003) had similarly found that fructose was approximately twice as
262 important as glucose in acrylamide formation in hash brown systems while fructose was significantly
263 more important than glucose in the formation of acrylamide in model systems (Pollien, Lindinger,
264 Yeretzian, & Blank, 2003). However, no significant difference in acrylamide formation between model
265 systems containing fructose, galactose, lactose and glucose were found (Stadler et al., 2002), while in
266 yeast-leavened wheat bread increased fructose did not significantly impact the formation of acrylamide
267 (Surdyk, Rosen, Andersson, & Aman, 2004). Furthermore, Parker et al. observed that glucose
268 concentration decreased twice as quickly as fructose concentration during the finish-frying of French
269 Fries but postulated that their relative reactivities would change when potato cultivars with lower amino
270 acid contents are studied. The corresponding moisture content at the end of the initial lag stage (80-
271 95 s) before acrylamide formation commences at 155 °C and when the potato slices undergo
272 dehydration (concentration of the reactants increasing) was determined experimentally to be
273 approximately 6% (dwb), which effectively coincides with the system reaching its isothermal state,
274 thereby obviating any need for complex heat and mass transport calculations during the effective

reaction period. The average moisture content at 155 °C (90 s) was 5.91 (n=12, sd 1.75) while similar results were obtained at 165 °C (n=22) and at 145 °C but at longer frying times (120 s, n=22). Although, the proposed kinetic model can only be phenomenological since the heat and mass transport behaviour during the early stages of frying is not yet understood, on the basis of the model shown in **Figure 4** and the kinetic data, the simplest rate equation for the formation of acrylamide is suggested as follows:

$$\frac{d[acryl]}{dt} = k_1[glu][TAA]\frac{[asn]}{[TAA]} + k_6[fru]\frac{[asn]}{[TAA]} \qquad (1)$$

Where [TAA] is the concentration of total amino acids. Thus, in the initial rate phase, the concentration of acrylamide formed after a time t is given by:

$$[acryl]_t = k_1[glu][asn]t + k_6[fru]\frac{[asn]}{[TAA]}t \qquad (2)$$

The critical variable is the reaction time t. The hypothesis to be tested here is that the formation of acrylamide begins at a 'critical' value of moisture content and that the effective reaction time may be calculated from the rate of dehydration at a given frying temperature and the final moisture content to be achieved.

Amino acid concentrations are grouped together as TAA. The validity of this approach was tested by including in the experimental protocol fortification of the potato slices with glutamine and glycine to determine any specific kinetic effects from these two amino acids with widely differing structures.

Model implementation. The initial concentrations of the major reactants were varied independently ([glucose]=32–217 mmol/kg, [fructose]=35–159 mmol/kg, [asn]=135–328 mmol/kg, [gln]=7–77 mmol/kg, [gly]= 0.1 mmol/kg and 48–164 mmol/kg, with [TAA]= 35–350 mmol/kg and [asn]/[TAA]=0.07–0.88) and the concentration of acrylamide was measured at 90, 120, 150 and 180 s frying time at oil temperatures nominally at 145, 155 and 165 °C. The moisture- and fat-contents of the fried product were determined and acrylamide concentrations and moisture levels were expressed on a dry weight non-fat basis. The reaction time was calculated by setting the critical moisture content to 6% (dwb) and calculating the time for the samples to reach the critical and final moisture contents using the 'quadratic formula' (with negative square root) to solve the quadratic in t. The reaction time was taken as the difference between the two calculated results. In the final calculation of the model, only data

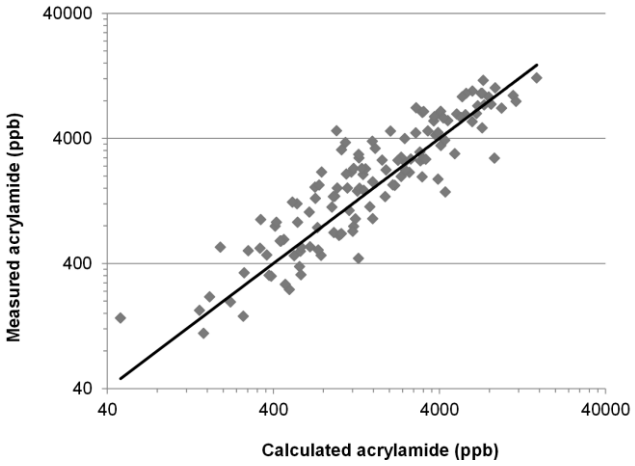
corresponding to a measurable rate of acrylamide formation, i.e., when the moisture content of the fried product was <c. 6% dry weight non-fat basis, were included.

The model was coded in Excel according to **Equation 2** with the addition of the temperature dependence of k_1 and k_6 according to the Arrhenius equation (**Equation 3**).

$$k_1 = A_1\, e^{-E_a/RT} \quad \text{and} \quad k_6 = A_6 e^{-E_a/RT} \qquad (3)$$

where A_1 and A_6 are the corresponding pre-exponential terms, E_a is the activation energy, R=8.314 J mol^{-1} K^{-1} and T is the absolute temperature.

The fit of the model to the experimental data is illustrated in **Figure 5** which spans a hundred-fold range in acrylamide yields. The model explains well the variation in acrylamide due to the experimental variables as illustrated by the plots of residuals.



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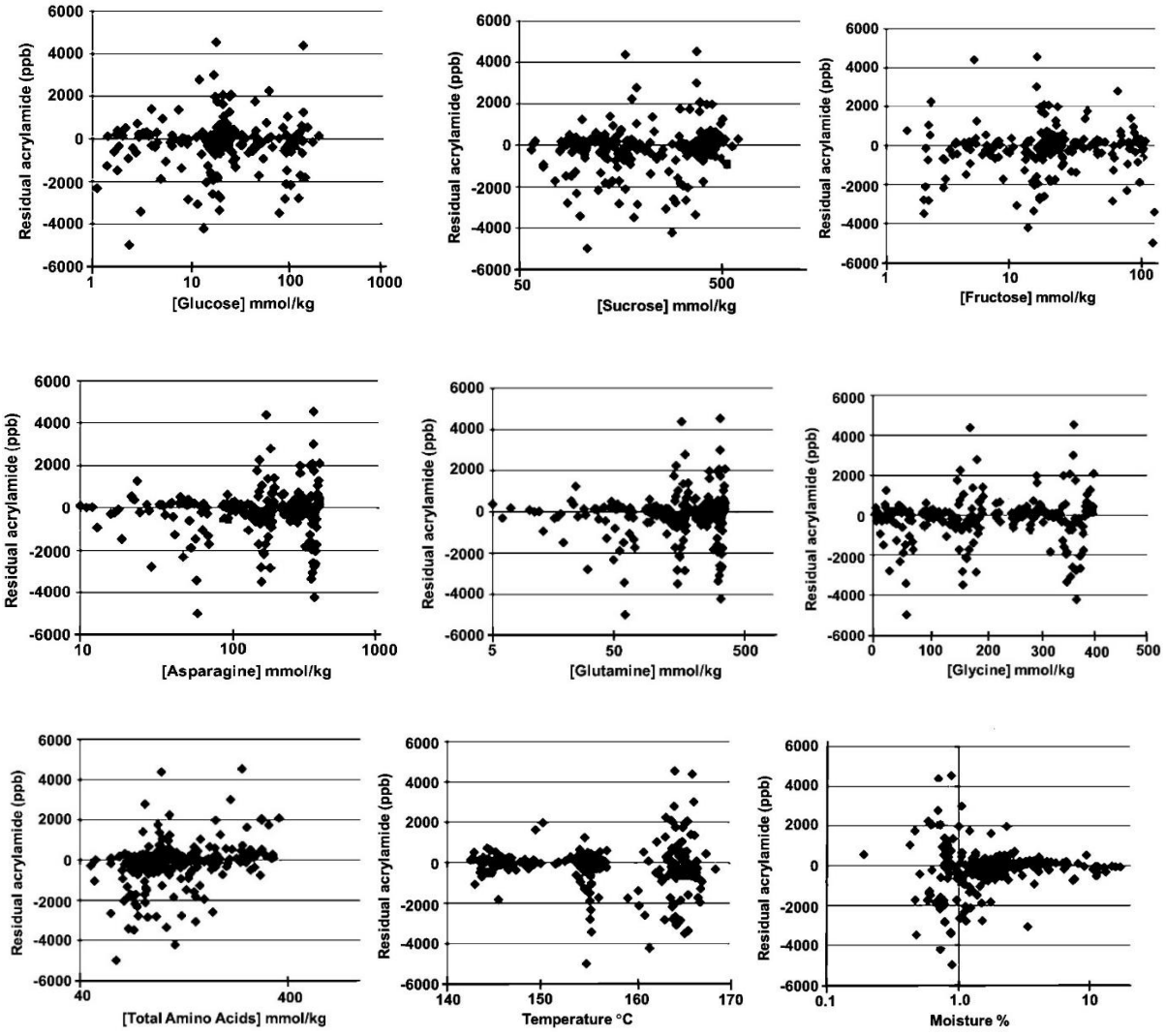

315 **Figure 5**. Fit of the kinetic model and residual plots given by equation 2 to kinetic data over a wide
316 concentration range of sugars and amino acids and at three frying temperatures (145, 155, 165 °C)
317 (*n*=214).

318 The kinetic parameters calculated for the data illustrated in **Figure 5** were A_1=1.29x10^{12} and
319 A_6=1.19x10^{10}, the pre-exponential terms corresponding to k_1 and k_6 (**equation 3**) and W_0 (6%) the
320 critical moisture content for the reaction to commence. Whereas the activation energy E_a (95 kJ/mol) is
321 assumed to be the same for the reactions of glucose and fructose, the model is unable to resolve the
322 contributions from the individual reactions.

323 The model depicted by **equation 1** is somewhat simpler than that proposed previously (Parker et al.,
324 2012) for the finish-frying of French fries, the latter comprising a number of consecutive rate-limiting

steps whereas **equation 1** is consistent with the occurrence of two parallel, single-step reactions. Whether or not the chemical mechanism for the formation of acrylamide is the same in French fries and PCs, dehydration processes and internal temperature profiles during the frying of PCs and French fries are very different hence, it is not surprising that the kinetics of acrylamide formation in the two processes differ.

CONCLUSIONS

The pre-treatment of raw potato slices using a combined blanching and fortification process successfully altered the chemical composition of the slices, providing a range of compositions suitable for kinetic studies. The relationship between the amount of solute dissolved in the blanching liquor and the uptake observed in the potato slices, after the 1 min exposure to the broth at 78 °C, was linear.

The rate of dehydration of PC with differing dry matter contents was investigated at specific intervals determined through the factorial experimental design. The data were used to formulate a quadratic equation for the time at which potato slices reached given moisture contents during frying and to calculate the effective reaction time, there being a critical moisture content of 6% (dwb) at which acrylamide formation began.

The chemical kinetic model was found to conform to the following rate equation:

$$\frac{d[acryl]}{dt} = k_1[glucose][asn] + k_6[fructose]\frac{[asn]}{[TAA]}$$

Where TAA represents the total amino acid concentration. The timescale of the frying process meant that the chemical reactions were all in their initial rate phase. The adoption of initial rates implies that initial concentrations were maintained throughout the observation period and is part of the overall hypothesis which is validated through modelling.

As a study in chemical kinetics, the range of reactant concentrations and ratios of [asn]/[TAA] were sufficiently large to elicit the specific behaviors of glucose and fructose, and of asparagine relative to the total amino acid pool. Thus, kinetic parameters confirm that the fructose-dependent reaction (caramelization) contributes twice as much acrylamide as the reaction of glucose (Maillard reaction).

Similarly, the fact that the outcomes of kinetic runs with modified concentrations of asparagine, glutamine and glycine can be predicted using the corresponding grouped concentration of amino acids implies that, within the accuracy of the available kinetic data, it is perfectly in order to treat the total amino acid concentration as a variable despite the species being made up of 20 or more amino compounds. We believe that this investigation is the most rigorous and detailed study of the kinetics of acrylamide formation in any food process and potentially provides the foundation for further optimizing acrylamide mitigation in PC according to the ALARA principle based on sugar and amino acid composition of potatoes.

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