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1   **Kinetic modelling of acrylamide formation during the finish-frying of**

2   **french fries with variable maltose content**

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Abstract

In light of a recent update in EU regulations governing levels of acrylamide in foodstuffs, further understanding of the role of different precursors is fundamental to extending mitigation strategies into a wider product range. Kinetic modelling was used to investigate the role of maltose in the formation of acrylamide during the finish-frying of french fries. The maltose concentration of raw white potato strips was systematically increased to observe the effect of this reducing disaccharide on acrylamide formation. A mathematical model, incorporating glucose, fructose and maltose and based on known Maillard reaction pathways, was developed which showed that acrylamide formation from maltose only contributed <10% to the total acrylamide. An additional kinetic model allowed for the formation of acrylamide directly from sugar-asparagine glycoconjugates. This model suggested that under these conditions, it is unlikely that acrylamide is formed directly from the maltose-asparagine conjugate.

1. Introduction

Since the discovery of high amounts of acrylamide, a probable carcinogen (IARC, 1994), in processed foods, there has been a considerable effort to reduce the acrylamide content in processed products either by manipulating the precursors and the processing conditions, or by obtaining raw materials with low asparagine content (Halford, Curtis, Muttucumaru, Postles, Elmore, & Mottram, 2012). In light of new EU regulations (Commision Regulation (EU) 2017/2158) which came into force in 2017 establishing tighter mitigation measures and benchmark levels for the reduction of acrylamide in food, a concerted approach to acrylamide mitigation is required. Further understanding of the role of different precursors under different conditions is fundamental to extending mitigation strategies into a wider product range.

Maltose is an important disaccharide found in many foods, and is formed from the breakdown of starch by β-amylases which are found in sweet potatoes, soya beans and germinating grains such as barley and wheat. It is a reducing sugar and our hypothesis is that maltose will contribute to the formation of acrylamide during high temperature processing of asparagine rich foods. Although the breakdown pathways of disaccharides during the Maillard reaction are well documented (Pischetsrieder & Severin, 1996; Smuda & Glomb, 2011), only a limited number of studies have focused on the role of disaccharides and their corresponding Maillard-derived intermediates in the formation of acrylamide (Koutsidis, De la Fuente, Dimitriou, Kakoulli, Wedzicha, & Mottram, 2008). In this study, we look at the relative contribution of maltose to acrylamide formation during a typical food manufacturing process and compare the relative rates of formation from glucose, fructose and maltose under different processing conditions. Combining established mechanisms for acrylamide formation from monosaccharides and for the maltose-induced Maillard reaction, this leads us to propose the following three possible pathways for maltose-induced acrylamide formation : 1) breakdown of maltose via the Maillard reaction to provide a pool of reactive carbonyl intermediates which subsequently react with asparagine to form acrylamide; 2) breakdown of

66 maltose to release glucose and subsequent formation of acrylamide from glucose and glucose

67 breakdown products; 3) direct breakdown of the maltose-asparagine glycoconjugate.

68 Multiresponse kinetic modelling is an effective technique to study the Maillard reaction

69 (Balagiannis, 2014; Parker, 2013; van Boekel, 2008). It can be used predictively to obtain

70 quantitative insight into complex reactions, but it can also serve as a means of probing and testing

71 different mechanistic pathways, thus providing strategies for controlling the reaction and, in this

72 case, minimising acrylamide. In particular, the kinetics of acrylamide formation has been studied in

73 simple mixtures of asparagine and reducing sugars, usually monosaccharides (De Vleeschouwer,

74 Van der Plancken, Van Loey, & Hendrickx, 2008a, 2008b, 2009a, 2009b; Knol, Linssen, & van

75 Boekel, 2010; Knol, Van Loon, Linssen, Ruck, van Boekel, & Voragen, 2005) under various

76 process conditions. Van der Fels-Klerx and coworkers (Nguyen, Van der Fels-Klerx, Peters, & van

77 Boekel, 2016; Nguyen, Van der Fels-Klerx, & van Boekel, 2017; Van Der Fels-Klerx et al., 2014)

78 extended this approach into biscuits, whilst Parker, Balagiannis, Higley, Smith, Wedzicha, &

79 Mottram (2012) used this approach in french fries taking into account the full complement of free

80 amino acids and reducing sugars in a complex food matrix. Parker et al. (2012) used a standard

81 protocol to manipulate the glucose and fructose levels in french fries, which were then processed in

82 a pilot-scale commercial fryer under different frying conditions. Uniquely, this model also

83 accounted for the moisture and heat transfer phenomena, as described for a one-dimensional infinite

84 slab by Ni and Datta (1999). Recently, this model was revisited, upgraded and extended to

85 approximate the mass and heat transfer events on a three dimensional basis (Balagiannis et al.,

86 2016).

87 In this study, the same process was used in order to investigate the role of maltose in acrylamide

88 formation during the finish frying of white potato fries. Although white potatoes are not a natural

89 source of maltose, they were selected for this study because the matrix can serve as a versatile

90 substrate in which to manipulate and study the effects of maltose content on the kinetics of

91 acrylamide formation. One further advantage was the fact that the behaviour of glucose and

92 fructose, and the heat and moisture transfer phenomena, have already been determined in white

93 potato fries (Parker et al., 2012), providing an ideal starting point for studying the relative

94 contribution of maltose, in comparison to glucose and fructose.

95 The aims of this study were to use multi response kinetic modeling to understand the role of

96 maltose in acrylamide formation in a real product, using French fries with variable maltose content

97 prepared under pilot-scale manufacturing conditions. By incorporation of similar data using glucose

98 and fructose from Parker et al. (2012), we developed one model that can explain and compare the

99 relative contribution from each of the sugars during acrylamide formation. Further to the role of

100 maltose, we also take the opportunity provided by this rich data set to develop a better

101 understanding of the mechanism of acrylamide formation by considering both the specific amino

102 acid pathway where reducing sugars react directly with asparagine to form acrylamide, and a

103 generic amino acid pathway where reactive precursors are formed via the Maillard reaction.

104 **2. Materials and methods**

105 **2.1 Materials**

106 Ranger Russet potatoes were obtained from the 2013 crop and stored at 7-9 °C until use. All

107 chemicals were supplied as described by Parker et al. (2012).

108 **2.2 Preparation of french fries with variable maltose content**

109 Ranger Russet potatoes were treated and processed as described by Parker et al. (2012) except that

110 the glucose and fructose dips were replaced by a maltose dip. The commercial production of french

111 fries involves a multi-step process which was reproduced as closely as possible on a pilot scale.

112 Briefly, the raw potatoes from harvest or storage were washed, peeled, preheated (54 °C, 30 min)

113 and cut into strips. The cross section of the raw potato strips was 7.5×7.5 mm and the length varied

114 between 7.5 and 12.5 cm. Subsequent blanching at 80 °C for 10 min causes some leaching of sugars

115 and was followed by a dipping step where the potato strips were immersed into a maltose solution

116 of varying concentration (70-75 °C, 30 s). The loss of sugars during blanching, and the requirement

117 to restore their concentration, is a part of the process that allows the control of the Maillard reaction,

5

118   thus influencing colour and acrylamide formation. The blanched potato strips were dipped in

119   maltose solutions of the following concentrations: 0% (only water), 1, 2, 3, 4 and 14% (w/v). The

120   outlying value of 14% was chosen to achieve a final maltose content similar to that of blanched

121   sweet potato strips. In sweet potatoes, the maltose increases up to ~2% during the manufacturing

122   process, depending on the variety and the processing conditions (Takahata, Noda , & Nagata, 1994).

123   Application of a 14% dip to white potato strips took the maltose content up to 1.4% (wet weight) in

124   the blanched strip. During blanching and dipping there was an uptake of ~10% of water which was

125   removed by a subsequent drying step (60 $^{\circ}$C, 10 min). The potato strips were then par-fried (190 $^{\circ}$C,

126   50 s) in canola oil, packaged and frozen (-20 $^{\circ}$C) before finish-frying in canola oil. Typically finish-

127   frying is carried out for 2-5 min, to an end point based on the desirable colour, texture, and flavour.

128   Each batch (~220 g) was finish-fried at 165 $^{\circ}$C for 10 different time points (0.5, 1.0, 1.5, 2.0, 2.5,

129   3.0, 3.5, 4.0, 4.5 and 5.0 min). Immediately after frying, the samples were submerged in liquid

130   nitrogen to prevent any further reaction, and stored at -20 $^{\circ}$C until further analysis. A similar

131   process was followed for a second batch of potatoes that was dipped in a solution of 2% maltose

132   and then fried at 175 and 185 $^{\circ}$C.

133   **2.3 Chemical analysis**

134   Acrylamide concentration was determined using the method described by Parker et al. (2012) using

135   an SPE clean-up procedure, analysis by LC-MS/MS and quantification using $^{13}C_3$-labeled

136   acrylamide as an internal standard.

137   The analysis of sugars was performed by HPLC according to the method reported by Parker et al.

138   (2012). The concentration of glucose, fructose, maltose, and sucrose in samples was calculated via

139   external calibration with authentic standards.

140   Free amino acids were measured via derivatisation as described by Parker et al. (2012). In

141   summary, the free amino acids Asn, Gln, Arg, Ala, Gla, Val, Asp, Ser, Lys, Phe, Tyr, Thr, Ile, Met,

142   Pro, His, Leu, and Gly were derivatised by *o*-phthalaldehyde and 9-fluorenylmethoxycabonyl

143   chloride in an autosampler immediately before injection in an Agilent HPLC 1100 series.

144 Analysis of water and total fat content were performed as described by Parker et al. (2012). Briefly,

145 moisture was determined by oven drying 2 g of sample until constant weight in a fan oven at 125

146 ℃. Fat was extracted, hydrolysed and methylated to produce fatty acid methyl esters which were

147 quantitated by GC-MS.

148 Colour formation was assessed with an Agtron E-30 Analyzer (Agtron Inc., Reno, NV) as described

149 in Parker et al. (2012).

150 **2.4 Kinetic modelling**

151 Multiresponse modelling and model simulations were performed using the Athena Visual Studio

152 software package (Athena Visual Software Inc., Naperville, IL).

153 **3. Results and discussion**

154 **3.1 General observations**

155 The maltose, glucose, fructose, sucrose, free amino acids and acrylamide contents were measured in

156 order to monitor the acrylamide formation in relation to the initial concentration of Maillard

157 reaction precursors. The data are all displayed graphically in supplementary Figure S1. The

158 concentration of sucrose did not alter during the course of the reaction (results not shown). The

159 temperatures that are typically applied during frying far exceed 100 ℃, which results in a

160 continuous evaporation and loss of moisture. Furthermore, the fried strips absorb oil during frying:

161 70-80% of the oil that is detected in the final finish-fried product adheres to the surface of the fry,

162 while the other 20-30% is oil that is trapped in the crust (Aguilera & Gloria-Hernandez, 2000). The

163 experimental data confirmed that the longer the frying time, the greater the loss of moisture and the

164 higher the amount of oil that was absorbed. In order to compare the trend of the components of

165 interest with time, it was necessary to normalise their concentration by removing any effect

166 introduced by their moisture and the fat content. Thus, throughout this paper, the concentration of

167 the analytes is reported on a defatted dry weight basis.

168 The experiment was performed with two different batches of white potatoes. In the first batch, the

169 concentration of maltose dip ranged from 0-14% and the fries were processed at 165 ℃ (samples

7

170   M0, M1, M2, M3, M4 and M14, Figure 1). In samples M0, M1, M2 and M3, all precursors

171   decreased over time, and there was a continuous increase in acrylamide. However, in samples M4

172   and M14, i.e. the two samples with the highest concentration of maltose, glucose either remained

173   stable (M4) or there was a small increase with time (M14). This is probably due to the formation of

174   glucose from the degradation of maltose, which in the samples with high maltose may occur faster

175   than the reaction of glucose with amino groups, thus the net result is a small accumulation of

176   glucose. The second batch of white potatoes was dipped in a 2% maltose solution and then samples

177   were finish-fried at either 175 $^{\circ}$C (M2'175) or 185 $^{\circ}$C (M2'185). These two potato batches had

178   different concentrations of precursors at the start: the raw fries in the first batch had on average 12.7

179   mmol/kg dry weight glucose and 131 mmol/kg dry weight of total free amino acids, whilst the raw

180   fries in the second batch had half as much glucose (6.1 mmol/kg dry weight) but almost double total

181   free amino acids (238 mmol/kg dry weight). These differences, whilst not immediately comparable

182   with the previous batch, provide additional information for the kinetic modelling and strengthen the

183   model.

184   Even though the maltose dip levels varied from 0% to 14%, the range of acrylamide formation was

185   narrow. Sample M0 reached a maximum of 5.4 μmol/kg dry weight, as a result of the reaction of

186   the endogenous fructose and glucose present in the potato strips. Sample M1 had a maximum of

187   13.4 μmol/kg dry weight, while the other samples (i.e. M2, M3, M4 and M14) had similar final

188   acrylamide concentrations in the range 20-24 μmol/kg dry weight. Beyond the 2% dip, further

189   increases in maltose concentration seem to have little impact on acrylamide formation, and this was

190   not due to exhaustion of the asparagine. In a similar experiment, when the glucose and fructose dips

191   varied up to just 2%, the final concentration of acrylamide was much higher, ranging from 8.2–86

192   μmol/kg dry weight (Parker et al., 2012). It is striking that the amount of acrylamide formed in the

193   batches with 2, 3, 4 and 14% maltose dip concentrations was similar even though it corresponded to

194   different degrees of maltose consumption (Table 1). For example, the fries with 2% and 14%

195   maltose dip showed similar acrylamide formation (20.1 and 20.8 μmol/kg dry weight respectively),

which corresponded to different losses of maltose (10.7 and 52.4 μmol/kg dry weight respectively).

Similarly, the study of the above systems in relation to colour formation (Table 1) showed that the fries with added maltose developed less colour than the systems with added glucose or fructose. In fact, after 5 min of frying, the samples which had been dipped in 14% maltose and 2% fructose had a similar colour, but M14 has consumed twice as much sugar but formed a fraction of the acrylamide.

It is likely that the maltose is converted into colourless higher molecular weight compounds. Frank and Hofmann (2000) have shown that colour formation in maltose is hindered by the 1,4-glycosidic link which is unhydrolysable under typical food preparation conditions. The maltose-derived glycosides are colourless compared to their monosaccharide counterparts which undergo further eliminations to generate coloured aglycones. Maltose also forms anhydrosugars, furosine and unreactive maltose specific compounds as suggested by Hollnagel and Kroh (2000) or other heterocycles with the 1-4 glycosidic link still intact, as shown by Kramhoeller, Pischetsrieder, & Severin (1993). Hollnager and Kroh (2000) report that the major intermediate formed from maltose degradation in a dry system at 100 °C was 1,4-didoxyhexosulose (1,4DDH) and this built up to concentrations of 18 mol% in relation to maltose. It is reactive in terms of condensations reactions, colour formation and the formation of heterocycles. However, they reported almost insignificant amounts of C2/ C3 α-dicarbonyls and α-hydroxycarbonyls, which may explain the formation of relatively little acrylamide. No-one as yet has shown the reactivity of 1,4DDH (an α-dicarbonyl) towards acrylamide formation. It seems that in french fries, the addition of maltose by dipping from the level of 2% onwards does not affect significantly the formation of acrylamide, at least not as much as it is affected by the addition of different levels of glucose or fructose shown by Parker et al., (2012).

To conclude, during frying of potato strips, maltose was less effective than glucose and fructose, in relation to the formation of Maillard products and acrylamide. This is in agreement with the fact that in Maillard browning, disaccharides are less reactive than monosaccharides (Lingnert, 1990;

222   Wedzicha & Kedward, 1995). In addition, Koutsidis et al. (2008) reported that maltose was less

223   potent than glucose and fructose with respect to acrylamide formation in a low-moisture waxy

224   maize starch model system that was heated at 160 ºC.

225   **3.2 Developing the kinetic mechanism**

226   Given the complexity of the reactions involved, one approach to gaining a better understanding of

227   these two pathways is to mathematically model the process based on the known chemistry of the

228   reactions. Modelling a complex reaction such as acrylamide formation is a challenging task. An

229   effective approach is to identify the few rate limiting steps that regulate the kinetic behaviour of the

230   reaction and develop a mathematical model based on them. As a result, the dense network of sub-

231   reactions that constitute a complex reaction are reduced to a limited number of kinetically important

232   steps. Each kinetic step incorporates several chemical pathways on the basis that the rate of a group

233   of reactions is determined by the speed of the slowest step. This approach has been applied

234   successfully on several occasions in the field of Maillard chemistry and acrylamide formation.

235   (Balagiannis et al., 2016; Balagiannis, Parker, Pyle, Desforges, Wedzicha, & Mottram, 2009;

236   Brands & van Boekel, 2002; De Vleeschouwer et al., 2008b; Knol et el., 2005; Low, 2006; Martins

237   & van Boekel, 2004; Parker et al., (2012); Wedzicha, Mottram, Elmore, Koutsidis, & Dodson,

238   2005). In earlier work on monosaccharides, we initially expressed the complex chemistry of

239   acrylamide formation in relation to glucose and fructose in a few kinetic steps which included both

240   the generic and specific amino acid pathways. However, there was high uncertainty in the kinetic

241   parameters, particularly those driving the split between the generic and specific amino acid

242   pathway, so a simpler model was published where these two pathways were collapsed into one,

243   incorporating two kinetically important intermediates (Parker et al., 2012). Subsequent iterations of

244   the model took into account the fact that more acrylamide is likely to be formed at the edges and

245   corners of the fries, as is observed anecdotally by the greater colour formation at the edges and

246   corners of the fries (Balagiannis et al., 2016).

247   For the purpose of the present study, the previously published kinetic mechanism (Balagiannis et

248   al., 2016) was the starting point for development of a new model incorporating maltose. Several

249   modelling trials indicated that the estimates could be improved if the transition from glucose to a

250   group of intermediates (Int2) was expressed by just one kinetic step instead of two. Furthermore, it

251   is well documented that Maillard reaction intermediates that contain an intact sugar backbone

252   fragment via retroaldolisation and oxidative fission reactions (Nursten, 2005) producing α-

253   dicarbonyls, α-hydroxycarbonyls or acids from the reducing terminus of the sugar, and tetroses and

254   trioses from the other end. These highly reactive compounds readily react with free amino acids.

255   This molar relationship was included in the updated kinetic mechanism and was expressed by

256   parameter p which reflects the fact that the number of moles of Int2 formed may be greater than the

257   number of moles of glucose lost. The new mechanism accounted for a larger consumption of free

258   amino acids during the reaction of Int2 to form acrylamide and Maillard products, removing the

259   necessity for the reaction previously associated with k_{aa} which had been included in previous

260   models to account for the undefined loss of free amino acids (Balagiannis et al., 2016; Parker et al.,

261   2012).

262   **3.3 Incorporation of maltose into the kinetic model**

263   The kinetic mechanism was then expanded to include the chemistry and the kinetics of acrylamide

264   formation in relation to maltose. This was achieved by taking into account the study by Mundt and

265   Wedzicha (2005). These authors modelled the formation of melanoidins in a solution of 0.2 M

266   sodium acetate/glacial acid buffer (pH 5.5), containing equimolar amounts of maltose (0.25 M) and

267   glycine, which was heated at 70 ºC for several time points. They followed a radiochemical approach

268   to propose a kinetic mechanism which described how maltose participates in the Maillard reaction.

269   In summary, they suggested that maltose reacts with glycine to form an intermediate which breaks

270   down to glucose and a second reactive intermediate, identified by Hollnager et al. (2000) as 1,4

271   DDH. The latter reacts further to form melanoidins via Int2. Hence, we propose Kinetic Mechanism

272   1 (Figure 2) which shows how maltose, glucose and fructose participate in the formation of

273   acrylamide. In Kinetic Mechanism 1 the role of maltose is dual: on the one hand, maltose itself (as a

274   reducing sugar) participates in the Maillard reaction and acrylamide formation, through a three-step

275   process and the formation of two pools of intermediates, $Int1_{mal}$ and Int (Pathway 1). On the other

276   hand, one glucose molecule is released from $Int1_{mal}$ through a "peel off" mechanism, (Mundt and

277   Wedzicha, 2005) and maltose contributes to the formation of acrylamide via the glucose and

278   fructose (Pathway 2). The following differential equations are derived from Kinetic Mechanism 1

279   (Figure 2) and comprise Model 1.

280    $$\frac{d[Glu]}{dt} = -k_1[Glu][FAA] + k_{2mal}[Int1_{mal}] - k_8[Glu]$$

281    $$\frac{d[Fru]}{dt} = -k_6[Fru] + k_8[Glu]$$

282    $$\frac{d[Mal]}{dt} = -k_{1mal}[Mal][FAA]$$

283    $$\frac{d[FAAs]}{dt} = -k_3[Int2][FAA]$$

284    $$\frac{d[Asn]}{dt} = -k_3[Int2][Asn]$$

285    $$\frac{d[Acr]}{dt} = k_3[Int2][Asn] * F_{Asn} * Asn$$

286    $$\frac{d[Int1_{mal}]}{dt} = k_{1mal}[Mal][FAA] - k_{2mal}[Int1_{mal}]$$

287    $$\frac{d[Int2]}{dt} = (1 + p)(k_1[Glu][FAA] + k_6[Fru] + k_{2mal}[Int1_{mal}]) - k_3[Int2][FAA]$$

288   where F_{Asn} is the fraction of asparagine that is converted to acrylamide. Note that during the

289   reactions associated with k_1 and k_{1mal}, the free amino acids are regenerated so there is no loss of free

290   amino acids associated with these pathways.

291   Athena Visual Studio was used to solve the simultaneous differential equations and estimate the

292   parameters using the data from the maltose-dipped fries, combined with the data from the fries with

293   variable glucose and fructose content taken from Parker et al. (2012). The parameter estimates are

294   shown in Figure 2. Overall, the parameters were good with respect to their 95% confidence

295 intervals. The fit of the model to the experimental values is very good, and for the maltose-dipped

296 samples this is demonstrated in Figure 1. The overall quality of the fit is shown in Figure 3, where

297 the observed vs. predicted plots include all the measured responses from all available datasets. In all

298 cases linear regression gave slopes close to 1.0 (0.96 to 0.103) and generally the R^2 was > 0.95. The

299 exceptions were for the free amino acids where there was more scatter, but the slope was still 1.0.

300 The estimate for parameter F_{Asn} is 32×10^{-4}, which implies that 0.3% of the asparagine is converted

301 to acrylamide. Also, parameter p was estimated as 0.85, which implies that from 1 mole of

302 intermediates with an intact sugar backbone, 1.85 moles of fragmentation products are formed.

303 Comparison of the kinetics of the formation of acrylamide from the three sugars was carried out in

304 Athena using model simulations. Using the rate constants from Figure 2, the model was run at 165

305 °C where the concentration of the total FAA and Asn were set to 164 and 60 mmol/kg dry weight

306 respectively (the same as in M0), and the initial acrylamide (generated during par-frying) was set to

307 zero. In turn, each of the sugars was set to 40 mmol/kg dry weight (falling within the experimental

308 ranges used) whilst the other two were set to zero, in order to determine the individual contribution

309 from each of the sugars. The simulations are shown in Figure 4a, and it is clear that maltose makes

310 only a small contribution to the total acrylamide formation. Figure 4b shows the data as a % of total,

311 where it is clear that fructose is initially faster than glucose under these particular conditions, and

312 the contribution from maltose increases with frying time to a maximum of 10% of the total.

313 However for the first 200 seconds, the contribution from maltose is less than 5%.

314 **3.4 Incorporation of specific and generic pathways into the kinetic model**

315 During food processing, two related pathways have been proposed for acrylamide formation (Parker

316 et al., 2012): the "specific amino acid pathway" and the "generic amino acid pathway". Whereas

317 Kinetic Mechanism 1 provides a very good model to describe the formation of acrylamide during

318 frying, it does not discriminate between the generic and the specific amino acid pathways.

319 The generic amino acid pathway involves the Maillard reaction (top line, Figure 5) - the reaction

320 between any of the free amino acids present in the food with reducing sugars to form a series of

13

321   Schiff bases. These rearrange to form the corresponding Amadori rearrangement products, and the

322   subsequent cascade of reactions results in the formation of highly reactive deoxyosones and short

323   chain carbonyl intermediates, particularly α-dicarbonyls such as glyoxal and methylglyoxal, α-

324   hydroxycarbonyls such as glycolaldehyde and hydroxypropanone, and also trioses and tetroses.

325   Many of these carbonyls react with amino acids more effectively than glucose does (Hofmann,

326   1999) and in particular with free asparagine to form acrylamide. This could be via the α-

327   hydroxycarbonyls as detailed by Stadler, Robert, Riediker, Varga, Davidek, Devaud et al. (2004)

328   (centre of Figure 5), or via α -dicarbonyls as shown by Koutsidis et al. (2008) and Amrein,

329   Limacher, Conde-Petit, Amado, & Escher (2006) (right-hand side of Figure 5). The chemistry of

330   this latter pathway has been less well defined but is certainly different when there is a vicinal

331   dicarbonyl involved. It may proceed via the 3-aminopropionamide, returning a dicarbonyl, or via β-

332   elimination (Stadler et al., 2004). In both cases, whether the reactive species is a dicarbonyl or a

333   hydroxycarbonyl, the Maillard reaction provides a source of those particularly reactive species that

334   asparagine requires in order to induce acrylamide formation. These pathways are certainly Maillard

335   assisted.

336   During the specific amino acid pathway, asparagine reacts directly with the reducing sugars present

337   in the food to form a sugar-asparagine glycoconjugate which dehydrates to a Schiff base (left-hand

338   side of Figure 5). This is followed by a series of reactions which involve the decarboxylation of the

339   Schiff base (thus by-passing the formation of the Amadori rearrangement product), and a

340   subsequent tautomerism which leads to the formation of acrylamide (Stadler et al., 2004). The

341   chemical mechanism involved in this pathway is the same as for the Maillard assisted pathway via

342   hydroxycarbonyls (Figure 5). However, the kinetics of this pathway are different, because it does

343   not require the initial formation of a source of reactive carbonyls. For the remainder of the kinetic

344   discussion, we will refer to this pathway as the specific sugar-Asn pathway.

345   It is important at this stage to highlight the difference between a chemical mechanism and a kinetic

346   mechanism. Chemically, what we have referred to previously as the specific amino acid mechanism

347 includes the reaction of asparagine directly with C6 reducing sugars and other α-hydroxycarbonyls

348 which are formed during the Maillard reaction (Stadler et al., 2004). However kinetically, the

349 specific reaction with Maillard-derived α-hydroxycarbonyls is effectively initiated by any amino

350 acid, and is therefore indistinguishable, from a kinetic point of view, from the generic amino acid

351 pathway. This Maillard-assisted specific amino acid pathway follows the chemistry of the specific

352 sugar-Asn pathway, but the kinetics of the general pathway.

353 Although the generic amino acid pathway involves several more steps, the reaction of asparagine

354 with the highly reactive carbonyl intermediates may be faster than the first step of the specific

355 sugar-Asn pathway. The proportion that each of these pathways contributes to the formation of

356 acrylamide in food is not yet clear, and is likely to depend on the composition of the raw material,

357 as discussed by Ngyuen et al. (2016).

358 Taking this into consideration, a new kinetic mechanism was proposed (Kinetic Mechanism 2,

359 Figure 1). The right-hand side of the mechanism expresses the specific sugar-Asn pathway, where

360 each sugar reacts with asparagine, and acrylamide is formed via a single kinetically important step.

361 The left-hand side of the mechanism expresses the generic amino acid pathway where acrylamide is

362 formed from the reaction of asparagine with a group of very reactive intermediates Int_2, as was

363 described in detail previously in this paper. Note that this also includes the reaction of reactive

364 hydroxycarbonyls via the Stadler chemical mechanism. The differential equations derived from

365 Kinetic Mechanism 2 (Model 2) are:

366 $$\frac{d[Glu]}{dt} = -k_1[Glu][FAA] + k_{2mal}[Int1_{mal}] - k_8[Glu] - k_g[Glu][Asn]$$

367 $$\frac{d[Fru]}{dt} = -k_6[Fru] + k_8[Glu] - k_f[Fru][Asn]$$

368 $$\frac{d[Mal]}{dt} = -k_{1mal}[Mal][FAA] - k_m[Mal][Asn]$$

369 $$\frac{d[FAAs]}{dt} = -k_3[Int2][FAA] - k_g[Glu][Asn] - k_f[Fru][Asn] - k_m[Mal][Asn]$$

370 $$\frac{d[Asn]}{dt} = -k_3[Int2][Asn] - k_g[Glu][Asn] - k_f[Fru][Asn] - k_m[Mal][Asn]$$

15

371 $$\frac{d[Acr]}{dt} = k_3[Int2][Asn] * F_{Asn} + k_g[Glu][Asn] + k_f[Fru][Asn] + k_m[Mal][Asn]$$

372 $$\frac{d[Int1_{mal}]}{dt} = k_{1mal}[Mal][FAA] - k_{2mal}[Int1_{mal}]$$

373 $$\frac{d[Int2]}{dt} = (1 + p)(k_1[Glu][FAA] + k_6[Fru] + k_{2mal}[Int1_{mal}]) - k_3[Int2][FAA]$$

374 This model contains three more parameters than the model 1. Athena Visual Studio was used to

375 solve the simultaneous differential equations and estimate the parameters. As for Model 1, the

376 combined data were used for parameter estimation giving rise to Model 2. The estimated parameters

377 of Model 2, as calculated by Athena Visual Studio, are shown in Figure 2. The fit of the model to

378 the experimental data is very good and similar to the fit of the model of Kinetic Mechanism 1. The

379 fits are shown in supplementary figures S2 and S3. Similar to Model 1, parameter *p* was estimated

380 as 0.66, which implies that from 1 mole of intermediates with an intact sugar backbone, 1.66 moles

381 of fragmentation products are formed. All the estimates for the reaction rate constants of Kinetic

382 Mechanism 2 were estimated with acceptable 95% confidence intervals (Figure 2) with the

383 exception of k_m which was returned as lower bound (zero). This shows that both generic and

384 specific sugar-Asn pathways are likely to contribute to the formation of acrylamide in french fries

385 and model simulations suggest that there is a significant contribution from both. Acrylamide is

386 formed via both routes when the participating sugars are glucose and fructose, but the formation

387 from maltose via the maltose glycoconjugate is kinetically insignificant and maltose contributes to

388 the generation of acrylamide only via the generic amino acid pathway or via glucose.

389 **3.5 Discussion of the chemical mechanisms**

390 We can draw some significant conclusions from the kinetic modelling. The data obtained could not

391 be fitted to the specific sugar-Asn route, but good models were obtained when the model was based

392 on the generic amino acid pathway (Model 1) and when we allowed the model to incorporate the

393 specific sugar-Asn route as well (Model 2). Although in modelling terms, Model 1 is preferable

394 because it is simpler, Model 2 shows that both routes are likely to contribute. To our knowledge,

395 this is the first kinetic model of acrylamide formation which has successfully incorporated both the

16

specific sugar-Asn and the generic amino acid pathways. Early models tended to favour the specific

sugar-Asn pathway as proposed by Stadler et al. (2004), and confirmed by Zyzak et al. (2003) who

showed evidence of the intermediate decarboxylated Amadori product in a high sugar system. Knoll

and coworkers modelled their data from aqueous buffered asparagine/glucose (Knol et al., 2005) or

asparagine/fructose (Knol et al., 2010) model systems using a two-step reaction based on the

specific amino acid pathway. This was successfully extended into low moisture systems by De

Vleeschouwer and co-workers (2008b, 2009b) who found that the best kinetic model fitted the

specific sugar-Asn pathway for acrylamide formation, and also included the Maillard reaction as a

browning pathway, but not as a source of reactive intermediates for acrylamide formation. This fit

is not unexpected from a system that contains just one amino acid and no other source of reactive

carbonyl species. However, this model also fitted when the data were collected from a potato-based

matrix (De Vleeschouwer et al., 2008a). Van der Fels-Klerx and co-workers (Nguyen et al., 2016,

2017) considered both the generic and specific sugar-Asn pathways when they developed their

model for acrylamide formation in biscuits, and again found the better models involved just

asparagine, although in their latest model, (Van Der Fels-Klerx et al., 2014) which incorporated the

participation of other free amino acids, the best model included the Maillard reaction as a sink for

the excess sugars, but not as a source of reactive intermediates for acrylamide formation. Wedzicha

et al. (2005) and Low, Mottram, & Elmore (2006) proposed a general amino acid pathway for the

formation of acrylamide and developed a kinetic model for the formation of acrylamide in low

sugar systems (potato, rye and wheat products). They observed the competition between the

formation of acrylamide and other Maillard-derived compounds, from a common pool of

intermediates, providing evidence for a significant contribution from the generic amino acid

pathway. In our earlier work (Parker et al., 2012), we initially considered both specific and general

amino acid pathways, but the model with the best fit was based on a three-step reaction relying on

the Maillard reaction to generate highly reactive intermediates (generic amino acid pathway) and

there were insufficient data to split the model into a generic and a specific component. In summary,

422   there is good evidence for both the existence of the specific sugar-Asn pathway in model systems

423   and biscuits, and the existence of the general amino acid pathway in cereals and potatoes. In this

424   paper, we have shown that these routes can co-exist during the frying of potato fries, and it is likely

425   that the contribution from the Maillard reaction depends certainly on the composition of the food,

426   and possibly on other factors. At one extreme, the glucose/asparagine model systems of Knoll and

427   co-workers (2010, 2005) were dominated by the specific pathway, since none of the particularly

428   reactive amino acids was present to promote the Maillard reaction and the formation of reactive

429   carbonyls. In the high sugar biscuit models (Nguyen et al., 2016, 2017) the molar ratio of reducing

430   sugars to asparagine was high (~50:1), and the contribution from the Maillard-assisted route may be

431   small enough that the best fit model is still based on the specific sugar-Asn pathway. However in

432   french fries, the molar ratio of reducing sugars to asparagine was low (~1:10), and model 2 suggests

433   a much greater contribution from the Maillard reaction. Under these conditions, the formation of

434   acrylamide is "Maillard assisted" by the accumulation of reactive carbonyl intermediates. This is an

435   important point to emphasise – the fact that the chemical mechanisms can change depending on the

436   composition of the food.

437   **4. Conclusion**

438   This is the first time that a holistic and robust model of acrylamide formation has been developed in

439   a real and complex food incorporating glucose, fructose and maltose, as well as moisture and heat

440   transfer parameters, and giving some insight into the relative contribution from different formation

441   pathways. At equimolar concentrations, maltose contributed < 10% to the total acrylamide

442   formation, giving us less concern over acrylamide levels in foods containing or generating maltose

443   (and by extension, higher oligomers of glucose). The first model demonstrates that the breakdown

444   of maltose and subsequent formation of acrylamide is relatively slow, whether this be via a maltose

445   Maillard intermediate route or via the "peel off" of glucose. The pathways from maltose require

446   three rate limiting steps as opposed to two from glucose or fructose. Model 2 which allowed for the

447   formation of acrylamide directly from sugar-asparagine glycoconjugates, showed there was a

448   contribution from the glucose and fructose glycoconjugates, but not from maltose glycoconjugate.

449   The more we understand about the formation pathways of acrylamide in real food systems, the

450   better we are equipped to develop appropriate mitigation strategies.

451   **Supporting information**

452   **Figure S1**. Graphs showing fit of all experimental data to model 1, generated from Kinetic

453   Mechanism 1.

454   **Figure S2**. Graphs showing fit of all experimental data to model 2, generated from Kinetic

455   Mechanism 2.

456   **Figure S2.** Graphs showing predicted vs. observed values for model 2, generated from Kinetic

457   Mechanism 2.

458 **5. References**

459 Aguilera, J. M., & Gloria-Hernandez, H. (2000). Oil absorption during frying of frozen parfried

460   potatoes. *J. Food Sci., 65*(3), 476-479.

461 Amrein, T. M., Limacher, A., Conde-Petit, B., Amado, R., & Escher, F. (2006). Influence of

462   thermal processing conditions on acrylamide generation and browning in a potato model

463   system. *J. Agric. Food Chem., 54*, 5910-5916.

464 Balagiannis, D. (2014). Predicting aroma formation with kinetic models. In J. K. Parker, J. S.

465   Elmore & L. Methven (Eds.), *Flavour Development, Analysis and Perception in Food and*

466   *Beverages*, (pp. 211-233). Cambridge, UK: Woodhead Publishing.

467 Balagiannis, D. P., Parker, J. K., Higley, J., Henson, T., Smith, G., Wedzicha, B. L., & Mottram, D.

468   S. (2016). A three dimensional kinetic model for the formation of acrylamide in french fries

469   with variable glucose and fructose content. In M. Granvogl, P. Schieberle & D. Peterson

470   (Eds.), *Browned Flavors: Analysis, Formation, & Physiology*, (pp. 67). Washington DC:

471   ACS.

472 Balagiannis, D. P., Parker, J. K., Pyle, D. L., Desforges, N., Wedzicha, B. L., & Mottram, D. S.

473   (2009). Kinetic modeling of the generation of 2- and 3-methylbutanal in a heated extract of

474   beef liver. *J. Agric. Food Chem., 57*, 9916-9922.

475 Brands, C. M. J., & van Boekel, M. A. J. S. (2002). Kinetic modeling of reactions in heated

476   monosaccharide-casein systems. *J. Agric. Food Chem., 50*, 6725-6739.

477 Commision Regulation (EU) 2017/2158 of November 2017, Establishing mitigation measures and

478   benchmark levels for the reduction of the presence of acrylamide in food. *Official Journal of*

479   *the European Union L304/24*.

480 De Vleeschouwer, K., Van der Plancken, I., Van Loey, A., & Hendrickx, M. E. (2008a).

481   Investigation of the influence of different moisture levels on acrylamide

482   formation/elimination reactions using multiresponse analysis. *J. Agric. Food Chem., 56*(15),

483   6460-6470.

484   De Vleeschouwer, K., Van der Plancken, I., van Loey, A., & Hendrickx, M. E. (2008b). The

485   kinetics of acrylamide formation/elimination in asparagine-glucose systems at different

486   initial reactant concentrations and ratios. *Food Chem., 111*, 719-729.

487   De Vleeschouwer, K., Van der Plancken, I., Van Loey, A., & Hendrickx, M. E. (2009a). Modelling

488   acrylamide changes in foods: from single-response empirical to multiresponse mechanistic

489   approaches. *Trends Food Sci. Technol., 20,* 155-167.

490   De Vleeschouwer, K., Van der Plancken, I., Van Loey, A., & Hendrickx, M. E. (2009b). Role of

491   precursors on the kinetics of acrylamide formation and elimination under low moisture

492   conditions using a multiresponse approach - Part II: Competitive reactions. *Food Chem.,*

493   *114*, 535-546.

494   Frank, O., & Hofmann, T. (2000). On the influence of the carbohydrate moiety on chromophore

495   formation during food-related Maillard reactions of pentoses, hexoses, and disaccharides.

496   *Helv. Chim. Acta, 83*, 3246-3261.

497   Halford, N. G., Curtis, T. Y., Muttucumaru, N., Postles, J., Elmore, J. S., & Mottram, D. S. (2012).

498   The acrylamide problem: a plant and agronomic science issue. *J. Exp. Bot., 63*(8), 2841-

499   2851.

500   Hofmann, T. (1999). Quantitative studies on the role of browning precursors in the Maillard

501   reaction of pentoses and hexoses with L-alanine. *Eur. Food Res. Technol., 209*, 113-121.

502   Hollnagel, A., & Kroh, L. W. (2000). Degradation of oligosaccharides in nonenzymatic browning

503   by formation of α-dicarbonyl compounds via a "peeling off" mechanism. *J. Agric. Food*

504   *Chem., 48*, 6219-6226.

505   IARC (1994). International Agency for Research on Cancer. IARC Monographs on the Evaluation

506   of Carcinogenic Risks to Humans. *60*, 389-433.

507   Knol, J. J., Linssen, J. P. H., & van Boekel, M. A. J. S. (2010). Unravelling the kinetics of the

508   formation of acrylamide in the Maillard reaction of fructose and asparagine by

509   multiresponse modelling. *Food Chem., 120*, 1047-1057.

510 Knol, J. J., van Loon, W. A. M., Linssen, J. P. H., Ruck, A.-L., van Boekel, M. A. J. S., & Voragen,

511   A. G. J. (2005). Toward a kinetic model for acrylamide formation in a glucose-asparagine

512   reaction system. *J. Agric. Food Chem., 53*, 6133-6139.

513 Koutsidis, G., De la Fuente, A., Dimitriou, C., Kakoulli, A., Wedzicha, B. L., & Mottram, D. S.

514   (2008). Acrylamide and pyrazine formation in model systems containing asparagine. *J. Ag.*

515   *Food Chem., 56*(15), 6105-6112.

516 Kramhoeller, B., Pischetsrieder, M., & Severin, T. (1993). Maillard reactions of lactose and

517   maltose. *J. Agric. Food Chem., 41*(3), 347-351.

518 Lingnert, H. (1990). Development of the Maillard reaction during food processing. In P. A. Finot,

519   H. U. Aeschbacher, R. F. Hurrel & R. Liardon (Eds.), *The Maillard Reaction in Food*

520   *Processing, Human Nutrition and Physiology*, (pp. 171-186). Basel: Birkhauser.

521 Low, M. Y. (2006). *Relationship between acrylamide formation and flavour generation in heated*

522   *foods.* Unpublished PhD Thesis, University of Reading, Reading, UK.

523 Low, M. Y., Mottram, D. S., & Elmore, J. S. (2006). Relationship between acrylamide formation

524   and the generation of flavour in heated foods. *Dev. Food Sci.,43*, 363-366.

525 Martins, S. I. F. S., & van Boekel, M. A. J. S. (2004). A kinetic model for the glucose/glycine

526   Maillard reaction pathways. *Food Chem.* , 257-269.

527 Mundt, S., & Wedzicha, B. L. (2005). Role of glucose in the Maillard browning of maltose and

528   glycine: A radiochemical approach. *J. Agric. Food Chem., 53*, 6798-6803.

529 Nguyen, H. T., Van der Fels-Klerx, H. J., Peters, R. J. B., & van Boekel, M. A. J. S. (2016).

530   Acrylamide and 5-hydroxymethylfurfural formation during baking of biscuits: Part I: Effects

531   of sugar type. *Food Chem., 192*, 575-585.

532 Nguyen, H. T., Van der Fels-Klerx, H. J., & van Boekel, M. A. J. S. (2017). Acrylamide and 5-

533   hydroxymethylfurfural formation during biscuit baking. Part II: Effect of the ratio of

534   reducing sugars and asparagine. *Food Chem., 230*, 14-23.

535   Ni, H., & Datta, A. K. (1999). Moisture, oil and energy transport during deep-fat frying of food

536   materials. *Food and Bioproducts Processing, 77*(3), 194-204.

537   Nursten, H. (2005). *The Maillard Reaction: Chemistry, Biochemistry and Implications*. Cambridge:

538   Royal Society of Chemistry.

539   Parker, J. K. (2013). The kinetics of thermal generation of flavour. *J. Sci. Food Agric., 93*(2), 197-

540   208.

541   Parker, J. K., Balagiannis, D. P., Higley, J., Smith, G., Wedzicha, B. L., & Mottram, D. S. (2012).

542   Kinetic model for the formation of acrylamide during the finish-frying of commercial

543   French fries. *J. Agric. Food Chem., 60*(36), 9321-9331.

544   Pischetsrieder, M. and Severin, T. (1996) Advanced Maillard reaction products of disaccharides:

545   analysis and relation to reaction conditions. In T.C. Lee & H,J, Kim (Eds.), *Chemical*

546   *Markers for Processed and Stored Foods*, (pp 14-23). Washington DC: ACS.

547   Smuda, M. & Glomb, M.A. (2011). Novel insights into the Maillard catalyzed degradation of

548   maltose. *J. Agric. Food Chem., 59*, 13254-13264.

549   Stadler, R. H., Robert, F., Riediker, S., Varga, N., Davidek, T., Devaud, S., Goldmann, T., Hau, J.,

550   & Blank, I. (2004). In-depth mechanistic study on the formation of acrylamide and other

551   vinylogous compounds by the Maillard reaction. *J. Agric. Food Chem., 52*(17), 5550-5558.

552   Takahata, Y., Noda, T., & Nagata, T. (1994). Effect of β-amylase stability and starch gelatinization

553   during heating on varietal differences in maltose content in sweetpotatoes. *J. Agric. Food*

554   *Chem., 42*, 2564-2569.

555   van Boekel, M. A. J. S. (2008). Multiresponse kinetic modeling of chemical reactions. In *Kinetic*

556   *Modeling of Reactions In Foods*. Boca Raton, FL,: CRC Press.

557   Van Der Fels-Klerx, H. J., Capuano, E., Nguyen, H. T., Atac Mogol, B., Kocadağli, T., Göncüoğlu

558   Taş, N., Hamzalioğlu, A., van Boekel, M. A. J. S., & Gökmen, V. (2014). Acrylamide and

559   5-hydroxymethylfurfural formation during baking of biscuits: NaCl and temperature-time

560   profile effects and kinetics. *Food Res. Int., 57*, 210-217.

561   Wedzicha, B. L., & Kedward, C. (1995). Kinetics of the oligosaccharide-glycine-sulfite reaction:

562   relationship to the browning of oligosaccharide mixtures. *Food Chem., 54*, 397-402.

563   Wedzicha, B. L., Mottram, D. S., Elmore, J. S., Koutsidis, G., & Dodson, A. T. (2005b). Kinetic

564   models as a route to control acrylamide formation in food. *Adv. Exp. Med. Biol.,*

565   *561*(Chemistry and Safety of Acrylamide in Food), 235-253.

566   Zyzak, D. V., Sanders, R. A., Stojanovic, M., Tallmadge, D. H., Eberhart, B. L., Ewald, D. K.,

567   Gruber, D. C., Morsch, T. R., Strothers, M. A., Rizzi, G. P., & Villagran, M. D. (2003).

568   Acrylamide formation mechanism in heated foods. *J. Agric. Food Chem., 51*, 4782-4787.

Table 1. Comparison of the rate of change of maltose and acrylamide in the maltose-dipped french fries and the 2% glucose-or fructose-dipped fries (data taken from Parker et al. (2012)) after finish frying at 165 °C for 5 min.

Addition level	**Maltose**						**Glucose**	**Fructose**
	0%	1%	2%	3%	4%	14%	2%	2%
Sug$_{ini}$[a]	-	16.4	24.2	41.6	47.3	164	63.0	72.8
Sug$_{fin}$[b]	-	7.1	13.5	20.7	26.3	111	27.3	50.4
Sug$_{ini}$-Sug$_{fin}$[c]	-	9.2	10.7	20.9	21.0	52.4	35.8	22.4
Acr$_{fin}$-Acr$_{ini}$[d]	4.5	10.9	20.1	17.5	19.4	20.8	72.7	75.4
Colour (Agtron Score)[e]	70.8	63.2	56.1	52.9	53.4	34.6	26.3	34.8

[a]concentration of the added sugar in fries before finish-frying (mmol/kg dry weight),
[b]concentration of the added sugar in fries after finish-frying (mmol/kg dry weight), [c]loss of added sugar (mmol/kg dry weight) during finish frying, [d]concentration of acrylamide in fries after finish frying (μmol/kg dry weight), [e]colour after finish frying
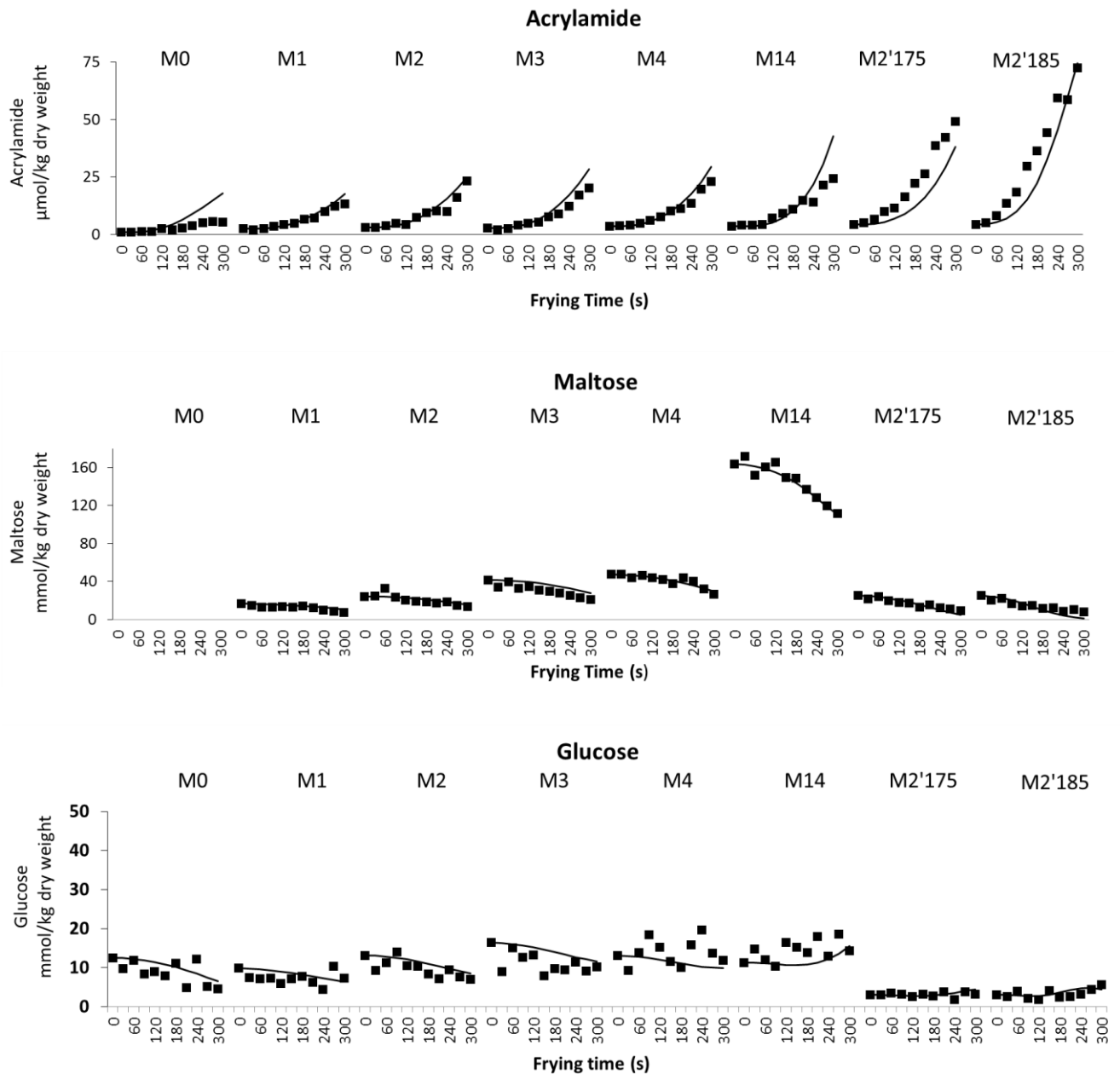
Figure 1. Acrylamide, maltose and glucose concentrations as a function of time (0−300 s) during finish-frying of eight batches of maltose-dipped potato strips at 165 ℃ (M0, M1, M2, M3, M4 and M14 represent 0, 1, 2, 3, 4 and 14% dip respectively), 175 ℃ (M2'175 = 2% dip) and 185 ℃ (M2'185= 2% dip). Symbols (■) are the experimental data points and the line (——) shows kinetic Model 1 derived from the combined data set using Kinetic Mechanism 1 (Figure 2).
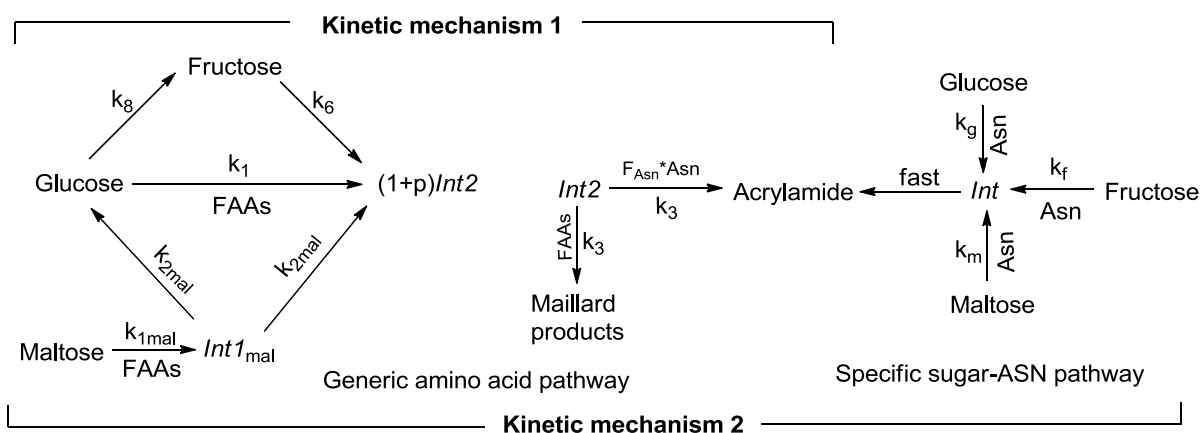
| Parameter	Model 1 based on **Kinetic Mechanism 1**		Model 2 based on **Kinetic Mechanism 2**	
	Optimal estimate x 10^4	95% Confidence interval x 10^4	Optimal estimate x 10^4	95% Confidence interval x 10^4
k_1 (mmol^{-1} kg s^{-1})	2.2	±0.03 (1%)	2.1	±0.3 (16%)
k_3 (mmol^{-1} kg s^{-1})	72	±25 (35%)	220	±62 (28%)
k_6 (s^{-1})	165	±9 (5 %)	161	±13 (8%)
k_8 (s^{-1})	68	±8 (12%)	73	±15 (20%)
p	8550	±2510 (29%)	6640	±2713 (41%)
F_{Asn}	32	±4 (14%)	18	±7 (38%)
k_{1mal} (mmol^{-1} kg s^{-1})	2.3	±0.1 (5%)	2.3	±0.1 (5%)
k_{2mal} (s^{-1})	162	±25 (15%)	245	±60 (25%)
k_g (mmol^{-1} kg s^{-1})			0.006	±0.003 (45%)
k_f (mmol^{-1} kg s^{-1})			0.005	±0.002 (40%)
k_m (mmol^{-1} kg s^{-1})			0 (lower bound)	-

Figure 2. Postulated Kinetic Mechanisms 1 and 2 with parameter estimates for Model 1 and Model 2 respectively, generated from data from fructose-, glucose- and maltose-dipped potato strips. The rate constants correspond to a temperature of 165 ºC.
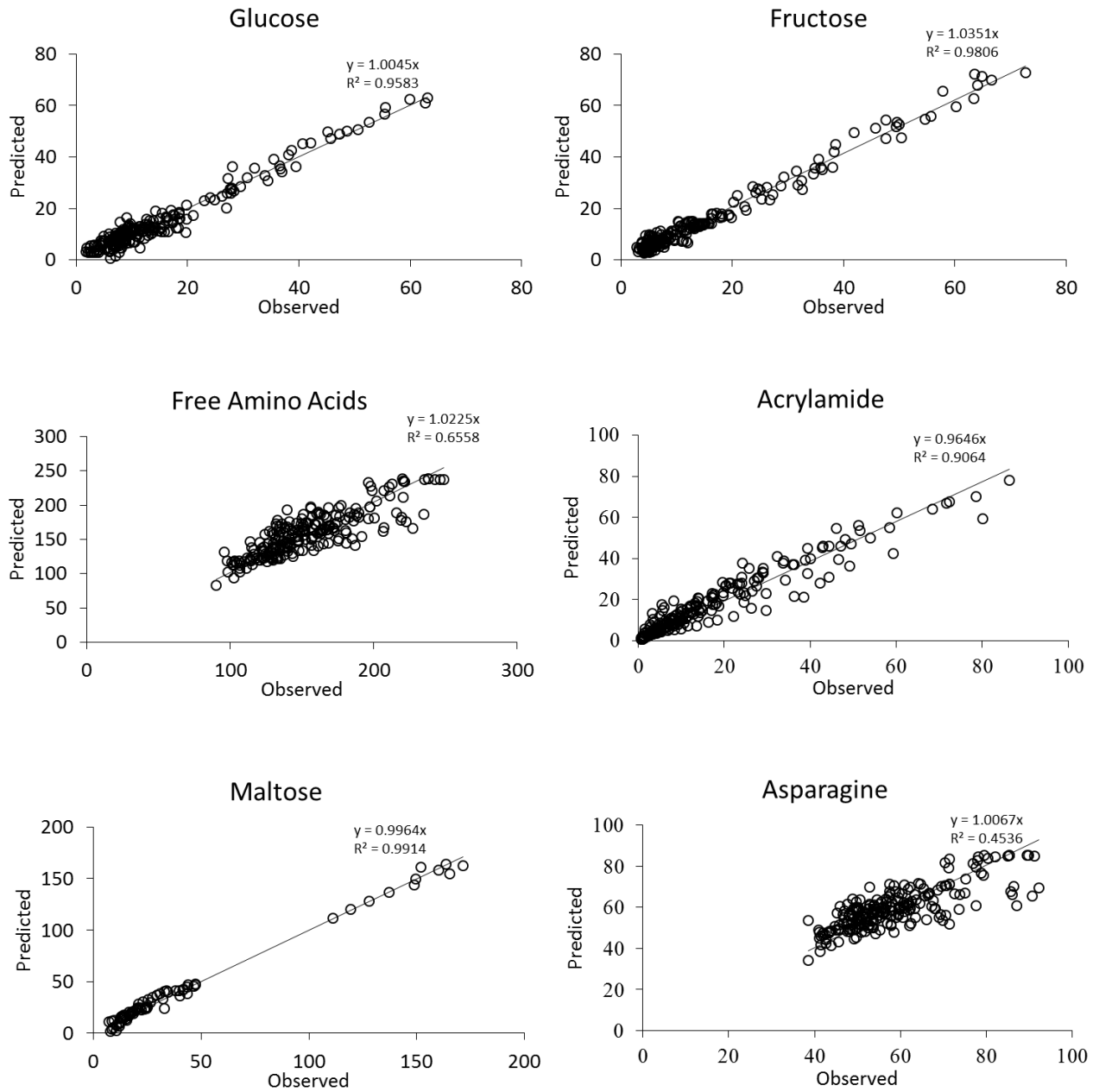
Figure 3. Predicted against observed values for Model 1 for all batches of fries compared with the line of perfect fit (y = 1). In all graphs the units are mmol/kg fat-free dry weight, except acrylamide which is expressed as μmol/kg fat-free dry weight. Linear regression was performed on each dataset to produce a slope (y) and R^2.
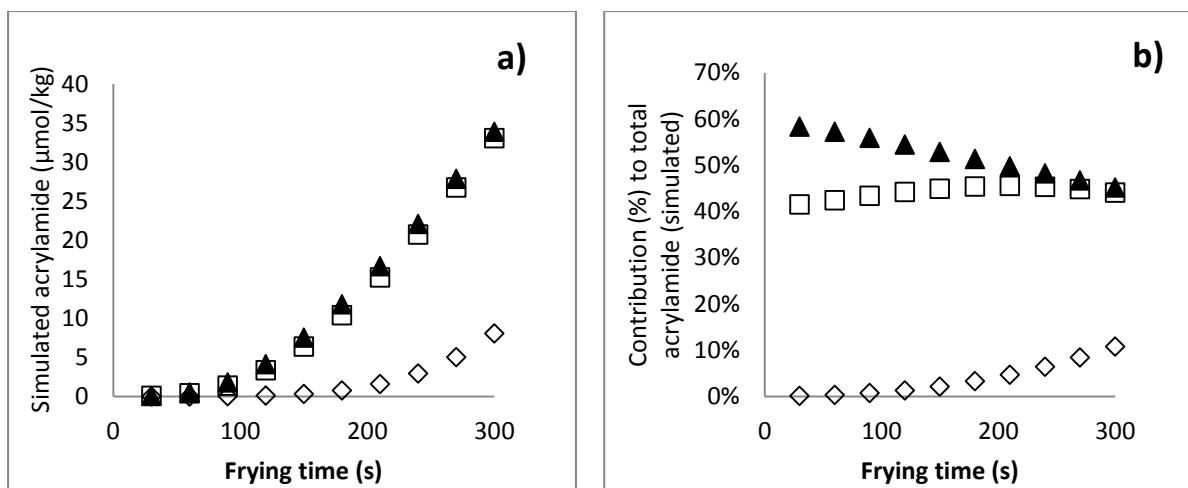
**Figure 4.** For Kinetic Mechanism 1, comparison of (a) the concentration of acrylamide formed and b) the % contribution to total acrylamide from glucose (□), fructose (▲), and maltose (◇) using model simulations at 165 °C where [FAA] at t = 0 was set to 146 mmol/kg dry weight, [Asn] to 60 mmol/kg dry weight, and the sugars were each set at 40 mmol/kg dry weight whilst the other two were set at 0.
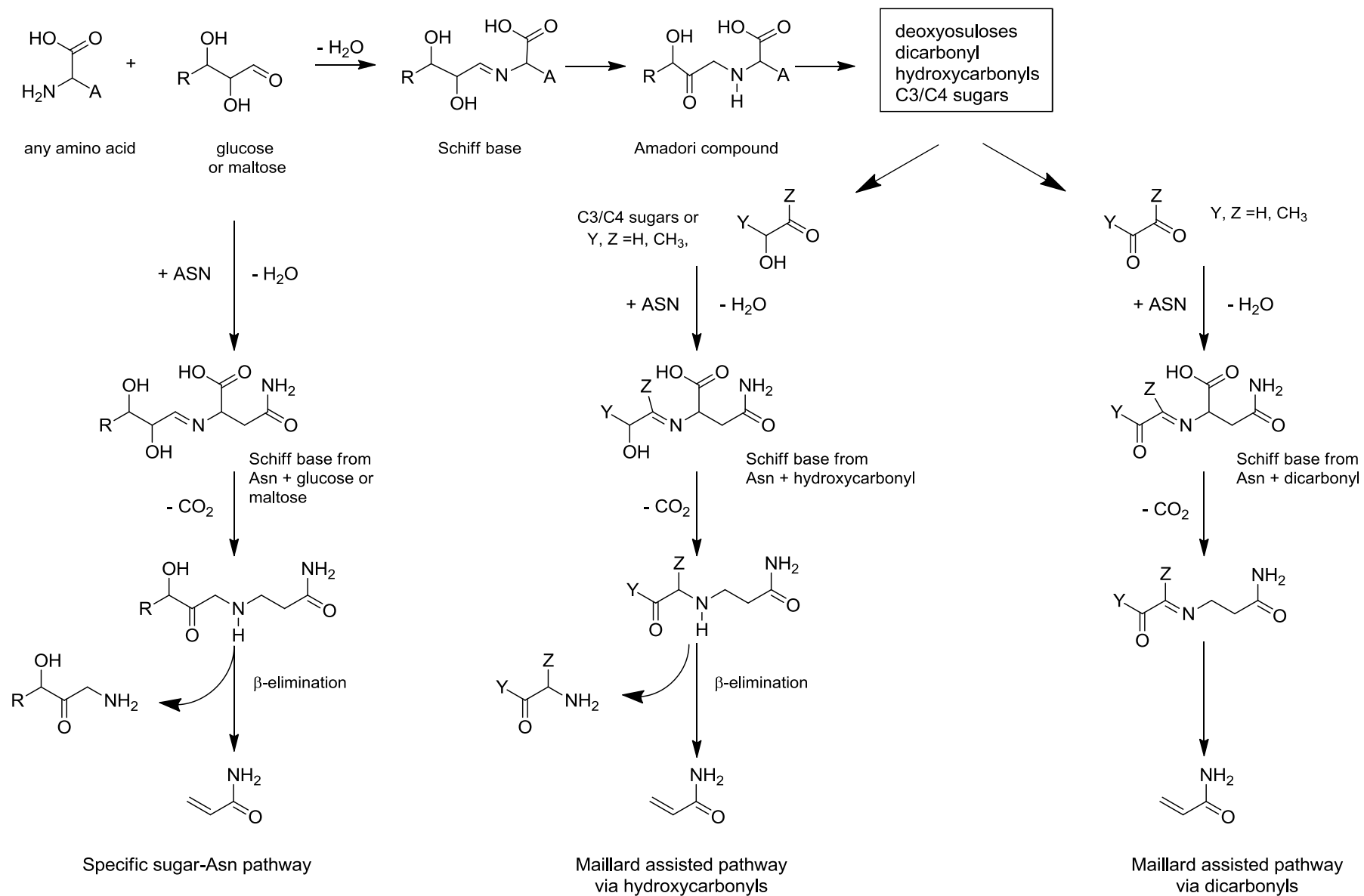
Figure 5. Proposed chemical mechanisms for the formation of acrylamide.
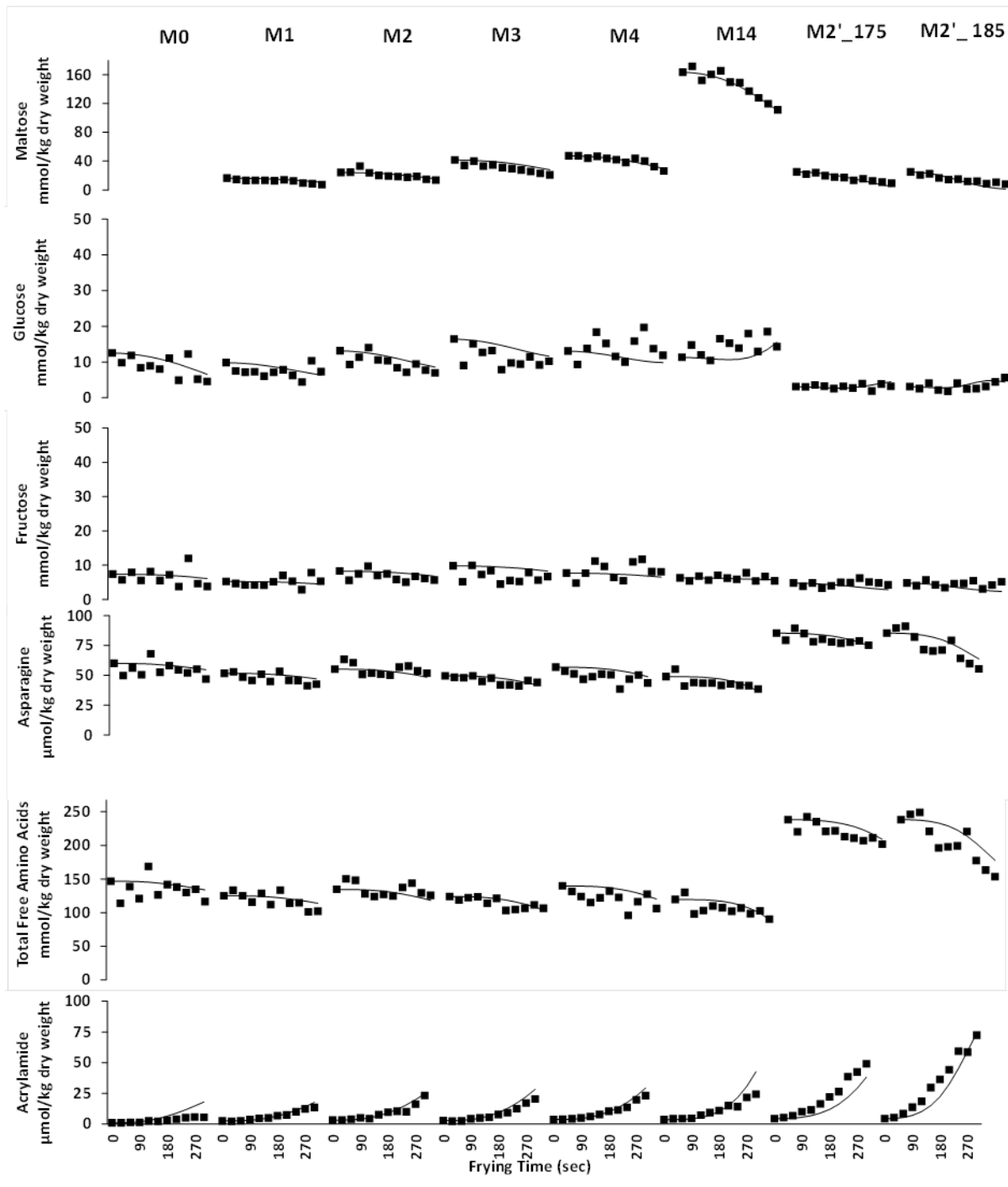
Figure S1 Maltose, glucose, fructose, asparagine, total free amino acids and acrylamide

concentrations as a function of time (0−300 s) during finish-frying of eight batches of

maltose-dipped potato strips at 165 °C (M0, M1, M2, M3, M4 and M14 represent 0, 1, 2, 3, 4

and 14% dip respectively), 175 °C (M2'_175 = 2.0% dip) and 185 °C (M2'_185= 2.0% dip).

Symbols (■) are the experimental data points and the line (——) shows the kinetic Model 1

derived from the combined data set using Kinetic Mechanism 1 (Figure 2).
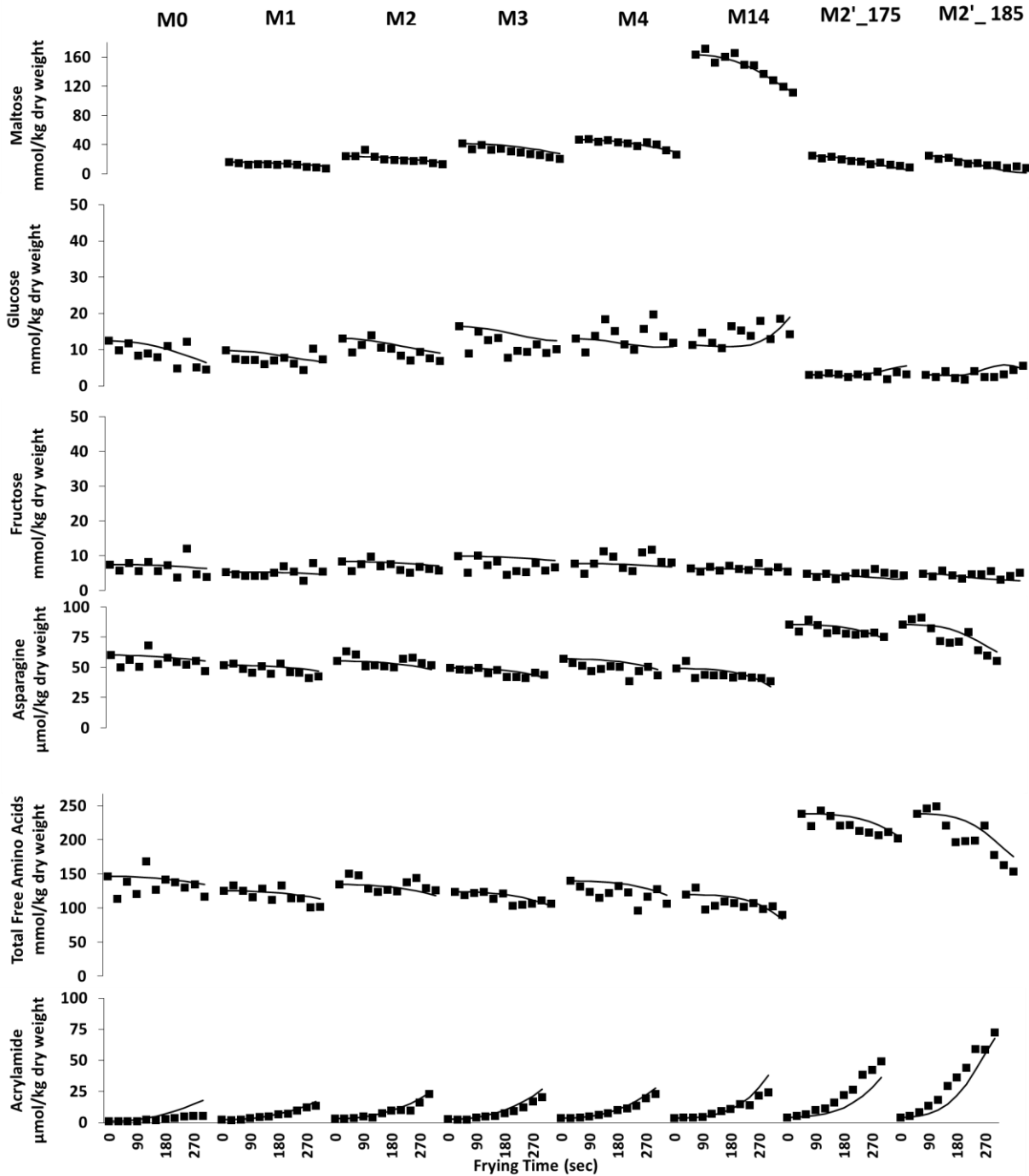
Figure S2. Maltose, glucose, fructose, total amino acids, and acrylamide concentrations as a function of time (0−5 min) during finish-frying of eight batches of maltose-dipped potato strips at 165 °C (M0, M1, M2, M3, M4 and M14 represent 0, 1, 2, 3, 4 and 14% dip respectively), 175 °C (M2'_175 = 2% dip) and 185 °C (M2'_185= 2% dip). Symbols (■) are the experimental data points, and the line (——) shows kinetic model Model 2 derived from the combined data set using Kinetic Mechanism 2 (Figure 2).
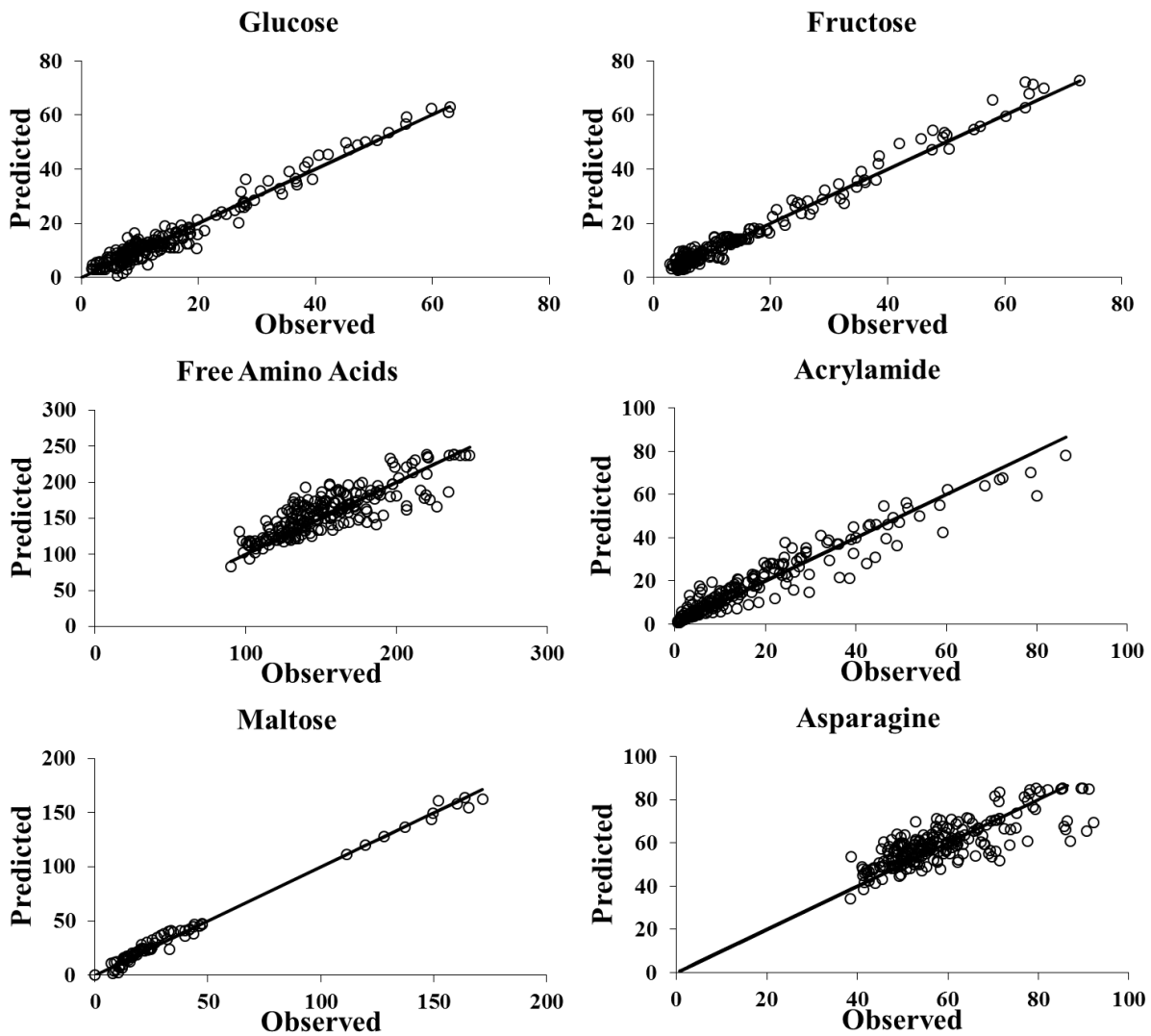
Figure S3. Predicted against observed values for Model 2 for all batches of fries compared with the line of perfect fit (y = 1). In all graphs the units are mmol/kg fat-free dry weight, except acrylamide which is expressed as μmol/kg fat-free dry weight.