Targeted precursor addition to increase baked flavour in a low acrylamide potato-based matrix

Article

Accepted Version

Kocadagli, T., Methven, L., Kant, A. and Parker, J. K. ORCID: https://orcid.org/0000-0003-4121-5481 (2021) Targeted precursor addition to increase baked flavour in a low acrylamide potato-based matrix. Food Chemistry, 339. 128024. ISSN 0308-8146 doi: 10.1016/j.foodchem.2020.128024 Available at https://centaur.reading.ac.uk/92872/

It is advisable to refer to the publisher's version if you intend to cite from the work. See Guidance on citing.

To link to this article DOI: http://dx.doi.org/10.1016/j.foodchem.2020.128024

Publisher: Elsevier

www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading

Reading's research outputs online

1   **Targeted precursor addition to increase baked flavour in a low acrylamide**

2   **potato-based matrix**

3

4   Tolgahan Kocadağlı[a,#], Lisa Methven[a], Avinash Kant[b], Jane K. Parker[a,*]

5   [a]Department of Food and Nutritional Sciences, University of Reading, Whiteknights, Reading RG6

6   6AP, UK

7

8   [b]PepsiCo International UK, Beaumont Park, Leycroft Road, Beaumont Leys, Leicester, LE4 1ET,

9   United Kingdom

10

11

12

13

14

15

16

17

18

19

20   *Corresponding author: Jane K. Parker

21   E-mail: j.k.parker@reading.ac.uk

22   Phone: +44 118 378 7455

23   #Current address: Department of Food Engineering, Hacettepe University, Ankara, Turkey

24

25   **Abstract**

26   The aim of this study was to increase the baked flavour of low acrylamide potato products. Strecker

27   aldehydes and pyrazines make an important contribution to the flavour of potato products and are

28   formed alongside acrylamide in the Maillard reaction. However, the Maillard reaction can be

29   directed in favour of aroma formation by selecting appropriate precursors and intermediates based

30   on the fundamental chemistry involved.

31   Selected precursors were added to potato dough prior to baking. Addition of glycine and alanine

32   together doubled high impact pyrazines and addition of 2,3-pentanedione or 3,4-hexanedione also

33   promoted the formation of key trisubstituted pyrazines. Quantitative descriptive profiling of

34   sensory attributes indicated that baked flavour was increased most when both Strecker aldehydes

35   and pyrazines were increased together. This work shows that it is possible to enhance baked flavour

36   in low acrylamide products by adding a specifically targeted combination of amino acids and key

37   intermediates, without increasing acrylamide concentration.

38

39   **Keywords: potato, complex matrix, acrylamide mitigation, flavour, Maillard, pyrazines**

40

1. Introduction

Fundamental research on exploring mitigation approaches for process contaminants, such as acrylamide, is an important area for the food & beverage industries. Specifically, greater understanding is required on maintaining the flavour quality of products as mitigation may alter flavour profiles. Model systems are useful approaches to study specific pathways, however this approach should also be supplemented with work on complex food systems to allow better translation into real food products. The use of simplified real food matrices is seen as a bridge between simple model systems and complex real food systems, and more studies using this approach can help in uncovering interaction and synergistic effects which may not occur in simple model systems.

High temperature thermal processing of asparagine rich foods results in the formation of acrylamide during the Maillard reaction (Mottram, Wedzicha, & Dodson, 2002; Stadler, Blank, Varga, Robert, Hau, Guy, et al., 2002) and it is classified as a Class 2a probable carcinogen in humans (International Agency for Research on Cancer, 1994). As flavour and browning also develop during this process, it is often a challenge to mitigate acrylamide formation from the viewpoint of food quality. Several strategies have been developed for this purpose however only a few of them mitigate acrylamide formation whilst allowing the cooked flavour and colour to develop. Selection of low sugar potato varieties and the use of asparaginase in bakery products have been proposed as the best mitigation strategies (Palermo, Gökmen, De Meulenaer, Ciesarová, Zhang, Pedreschi, et al., 2016).

Lowering thermal input is a convenient approach for baked foods for the mitigation of acrylamide formation (Gokmen, Acar, Koksel, & Acar, 2007; Taeymans, Wood, Ashby, Blank, Studer, Stadler, et al., 2004), but as this strategy depends on limiting the overall Maillard reaction, the final food product lacks baked flavour.

64   A pool of reactive intermediates is formed from the reaction between sugars and amino acids in the

65   intermediate stage of the Maillard reaction (Yaylayan, 1997). The composition of this pool

66   determines the flavour and browning characteristics of the final product (Yaylayan, 2003) and also

67   influences acrylamide formation. The baked flavour is largely obtained by Strecker aldehydes and

68   trisubstituted alkylpyrazines which are formed from the intermediates found in the pool. Addition

69   of amino acids and other precursors can be used to alter the composition of the pool to manipulate

70   flavour formation.

71   Strecker aldehydes are formed from free amino acids during the Maillard reaction via a number of

72   different pathways (Yaylayan, 2003). In particular, α-dicarbonyl compounds transform amino acids

73   to their Strecker aldehydes via decarboxylation. Strecker aldehydes which are important for aroma

74   are 2-methylpropanal (malty), 2-methylbutanal (malty, cacao like), 3-methylbutanal (malty, cacao

75   like), methional (boiled potato) and phenylacetaldehyde (floral, honey like) and they are formed

76   from valine, isoleucine, leucine, methionine and phenylalanine, respectively (Martin & Ames, 2001).

77   In addition, the Strecker aldehydes formaldehyde and acetaldehyde, formed from glycine and

78   alanine respectively, contribute to the pool of intermediates and can be precursors of methyl and

79   ethyl substituted pyrazines, respectively (Amrani-Hemaimi, Cerny, & Fay, 1995; Low, Koutsidis,

80   Parker, Elmore, Dodson, & Mottram, 2006).

81   α-Dicarbonyl compounds are converted into α-amino carbonyl compounds during the Strecker

82   degradation of amino acids, whereas the reaction of α-hydroxycarbonyl compounds and ammonia

83   may produce α-aminocarbonyl compounds via Amadori rearrangement (Yaylayan, 2003). N-

84   terminal amino acids in peptides have also been shown to transform α-dicarbonyl compounds into

85   their α-amino carbonyl derivatives with a different reaction mechanism to Strecker degradation

86   (Van Lancker, Adams, & De Kimpe, 2010, 2012). Condensation of two α-amino carbonyl compounds

87   produces a dihydropyrazine and subsequent oxidation yields a pyrazine (Shibamoto & Bernhard,

88 1977). Methyl substitution in pyrazines may originate from methylglyoxal (via 2-aminopropanal or

89 1-amino-2-propanone), 1-hydroxy-2-propanone (via 2-aminopropanal), 2,3-butanedione and 3-

90 hydroxy-2-butanone (via 3-amino-2-butanone) followed by oxidation of the corresponding

91 dihydropyrazines (Chu & Yaylayan, 2009). Ethyl substitution may come from 2-oxobutanal, 2,3-

92 pentanedione and 3,4-hexanedione via this oxidative pathway (Chu & Yaylayan, 2009). In addition

93 to this oxidative pathway, nucleophilic attack by a dihydropyrazine at the carbonyl centre of

94 formaldehyde or acetaldehyde, followed by the elimination of a water molecule, also yields alkyl

95 substituted pyrazines in a non-oxidative way (Amrani-Hemaimi, Cerny, & Fay, 1995; Cerny & Grosch,

96 1994).

97 The pyrazines which we are targeting in this work are the trisubstituted alkylpyrazines with relatively

98 low odour thresholds and baked aroma (2-ethyl-3,5-dimethylpyrazine, 2-ethyl-3,6-

99 dimethylpyrazine and 2,3-diethyl-5-methylpyrazine) since they are known to contribute to the

100 baked flavour in potato products (Buttery, Seifert, Guadagni, & Ling, 1971; R. Wagner, Czerny,

101 Bielohradsky, & Grosch, 1999; R. K. Wagner & Grosch, 1998). On the basis of the chemistry

102 underpinning the formation of these baked flavour compounds, we propose that with addition of a

103 carefully targeted selection of precursors to a potato-based matrix, we will be able to increase the

104 formation of key aroma compounds whilst maintaining low levels of acrylamide. Glycine and alanine

105 were selected to increase overall Maillard reaction and also as alkylpyrazine precursors. Leucine,

106 isoleucine, valine, phenylalanine, and methionine were selected as Strecker aldehyde precursors.

107 2,3-Pentanedione (PD) and 3,4-hexanedione (HD) were used as intermediates in alkylpyrazine

108 formation. Moreover, the addition of whey protein hydrolysate was assessed, also together with

109 2,3-pentanedione and 3,4-hexanedione, as oligopeptides have previously been shown to be very

110 effective in alkylpyrazine formation (Scalone, Lamichhane, Cucu, De Kimpe, De Meulenaer, 2019).

111 **2. Materials and Methods**

2.1 Chemicals and Consumables

$^{13}C_3$-Acrylamide was purchased from Cambridge Isotope Laboratories (QMx, Thaxted, UK). Acetonitrile (LC-MS grade), NaCl (reagent grade) and formic acid (LC-MS grade) were purchased from Fisher Scientific (Loughborough, UK). Deionised water was supplied from a MilliQ system at 18.2 MΩ.cm resistivity. Food grade amino acids were supplied from S.A. Ajinomoto OmniChem N.V. (Wetteren, Belgium). Amino acids reference mix solution (2.5 mM each), 2,3-pentanedione (food grade) and 3,4-hexanedione (food grade) were purchased from Sigma-Aldrich (Poole, UK).

2.2 Preparation of Potato-Based Matrix

A potato-based dough was prepared by mixing 342 g potato flake, 90 g waxy maize starch, 4.5 g lecithin, 4.5 g NaCl, 9 g sucrose and 300 g tap water at room temperature. Sucrose and NaCl were dissolved in water for the control formula. Amino acids, 2,3-pentanedione and 3,4-hexanedione were also dissolved in water to incorporate them homogenously into the dough. The amount of added amino acids was four times that of the naturally occurring levels in the potato flake (Table S1, see supplementary material) for all amino acids except Gly. Gly was added at 20- or 100-fold the naturally occurring level in the potato flake. Additionally, the 'all amino acids' recipe contained twice that of the naturally occurring concentrations of each amino acids except asparagine and γ-aminobutyric acid which were omitted from the mix. 2,3-Pentanedione and 3,4-hexanedione were added at 100 mg/kg on a dry basis. Whey protein hydrolysate was added at 5% on a dry basis. Dry ingredients were mixed on setting 1 (Kenwood Mixer) for 30 s and then the aqueous mixture was gradually added during mixing for 1 min. The dough was sheeted by using a pastry sheeter (Rondo, Burgdorf, Switzerland) to a thickness of 1.2 ± 0.06 mm. The dough was cut into disks of 5 cm and the pieces were pinned. Twenty-four pieces were transferred onto an open mesh baking mat and were baked at 160 °C for 6 min followed by drying at 130 °C for 10 min under full convection mode.

135   They were cooled down for 5 min on an aluminium foil at room temperature and immediately

136   packed in foil pouches by heat sealing and kept at -20 °C until chemical or sensory analysis.

137   ***2.3 Amino Acids Analysis***

138   Ground potato flake (1.0±0.05 g) was extracted with water in three stages using 10, 5 and 5 mL

139   respectively. At each stage, the tube was vortexed for 5 min followed by centrifugation at $6654 \times g$

140   for 5 min. Supernatants were combined in a tube, and 0.2 mL of combined extract was mixed with

141   0.8 mL acetonitrile. The mixture was centrifuged and a part of the supernatant was filtered through

142   0.2 µm PTFE filter (Fisher Scientific, UK) into an autosampler vial. External calibration curves of

143   amino acids were built between 1-50 µM in water:acetonitrile mixture (2:8, v:v).

144   Amino acids were analysed using an Agilent 1200 high-performance liquid chromatography (HPLC)

145   system coupled to a 6410 triple quadrupole mass spectrometer with electrospray ion source (ESI)

146   in positive mode. Chromatographic separation was carried out on a Synchronis HILIC column (150 ×

147   4.6 mm i.d., 3 µm) with a Synchronis HILIC precolumn (10 × 4.6 mm i.d., 3 µm, ThermoFisher

148   Scientific, Waltham, MA, USA). The mobile phase A was 5 mM ammonium formate with 0.5% formic

149   acid in water, and B was 5 mM ammonium formate with 0.5% formic acid in acetonitrile:water (9:1,

150   v:v) at a flow rate of 1 mL/min at 20 °C with gradient elution. Mobile phase A was increased from

151   10% to 40% for 8 min and then decreased to 10% in 1 min and kept at 10% for 4 min. The total run

152   time was 13 min. The injection volume was 5 µL. ESI source had the following settings: gas

153   temperature 330 °C, gas flow 13 L/min, nebuliser pressure 40 psi, capillary 4000 V. Amino acids

154   were identified by multiple reaction monitoring (MRM) using the ion transitions and parameters

155   previously reported (Kocadağlı, Özdemir and Gökmen, 2013).

156   ***2.4 Acrylamide Analysis***

157 The ground sample (1±0.05 g) was put into 50 mL centrifuge tube and 2 mL 1 mg/L $^{13}C_3$-acrylamide

158 was added followed by 38 mL water. After vortexing for 20 min, the tubes were centrifuged at

159 8422×g for 15 min at 15 °C. An aliquot of the supernatant was filtered through a 0.2 μm nylon

160 syringe filter (Fisher Scientific, Loughborough, UK) into an autosampler vial. An external calibration

161 curve of acrylamide was built between 0.5-20 μg/L, each containing 50 μg/L $^{13}C_3$-acrylamide.

162 Samples were analysed using an Agilent 1200 high-performance liquid chromatography (HPLC)

163 system coupled to a 6410 triple quadrupole mass spectrometer with electrospray ion source (ESI)

164 in positive mode. Chromatographic separation was carried out using a Hypercarb column (100 × 3.0

165 mm i.d., 5 μm) with a Hypercarb precolumn (10 × 3.0 mm i.d., 5 μm, ThermoFisher Scientific,

166 Waltham, MA, USA). The mobile phase was 0.1% aqueous formic acid at a flow rate of 0.3 mL/min

167 at 30 °C. The injection volume was 5 μL. ESI source had the following settings: gas temperature 350

168 °C, gas flow 10 L/min, nebuliser pressure 35 psi, capillary 3000 V and fragmentor voltage 40 V. The

169 MRM transitions of m/z 72 → 55 (N_2 collision energy (CE), 8 eV) and 72 → 27 (CE, 16 eV) were

170 measured for acrylamide, and the MRM transition of m/z 75 → 58 (CE, 8 eV) was measured for $^{13}C_3$-

171 acrylamide. A dwell time of 100 ms was set for all three MRM transitions.

172 **2.5 Aroma Analysis**

173 Volatile compounds were collected onto Tenax TA using dynamic headspace extraction. The cooked

174 potato-based matrix was ground and 10 g was transferred into a 250 mL conical flask with a screw-

175 thread neck and 15 mL saturated NaCl solution (35 g/100 mL) was added. The flask was kept at 50

176 °C in a water bath during the extraction. Oxygen-free nitrogen was passed over the sample for 1 h

177 at a rate of 40 mL/min, carrying volatiles onto a preconditioned glass trap (105 mm × 3 mm i.d.)

178 containing Tenax TA (85 mg) (SupelCo, Poole, UK). At the end of the collection, 130.6 ng of 1,2-

179 dichlorobenzene in 1μL methanol was injected onto the trap as an internal standard. Then, the trap

180 was flushed with nitrogen at a flow rate of 100 mL/min for 10 min to remove water. Volatiles

181 collected on the trap were desorbed using a Turbomatrix ATD system (Perkin Elmer, Beaconsfield,

182 UK) coupled to an Agilent GC-MS system (7890A-5975C). The Tenax traps were desorbed at 300 °C

183 (heating rate 40 °C/s) and cryofocused in a cold trap at −30 °C. Chromatographic separation was

184 achieved on a Phenomenex ZB-Wax column (30 m × 250 μm i.d.× 1 μm). The time-temperature

185 program employed was 10 min at 30 °C, a ramp of 4 °C/min to 250 °C, and hold at 250 °C for 10 min.

186 The carrier gas was He at 1 mL/min. The mass spectrometer was operated in electron ionization

187 mode with an ionizing voltage of 70 eV and source temperature of 200 °C. A scan range of m/z 29-

188 450 was employed with a scan time of 0.7 s. The data were controlled and stored using ChemStation.

189 A series of n-alkane standards (C_5-C_{25}) in diethyl ether was run to obtain linear retention index (LRI)

190 values. The identity of the compounds was confirmed by comparing their mass spectra with NIST

191 Mass Spectral Database, authentic compounds and LRI values (Table S2 & S3, see supplementary

192 material). The compounds were semi-quantified by comparing the peak areas of extracted ion

193 chromatograms with that of the internal standard 1,2-dichlorobenzene, using a response factor of

194 1.

195 ***2.6 Sensory Analysis***

196 A trained in-house panel of 11 assessors (10 female, 1 male) were used to estimate sensory changes

197 quantitatively. Samples were presented to assessors with random codes to discuss as a group, with

198 the help of a panel leader, and to develop a consensus vocabulary on the sensory attributes of the

199 cooked potato-based products. References (real food or aroma compounds) were available for the

200 panel to help define the product attributes during four sessions of 1 h (Table S4, see supplementary

201 material). During development of the sensory attributes, panellists were first asked to sniff and then

202 bite into the sample to define aroma, taste, flavour and aftertaste attributes. The day before the

203   quantitative sensory assessment took place, the panellists were exposed again to the references

204   and samples to standardise the vocabulary associated with defined attributes (30 min). Panellists

205   were asked to score the attributes of the control sample during the training session to obtain

206   average scores. The quantitative sensory assessments were carried out in well-ventilated individual

207   sensory booths controlled at 21±1 °C, in duplicate (replicates were presented on consecutive days).

208   The panellists were given the average scores together with the control sample (introduced with a

209   code 'reference') during the quantitative sensory assessments. Panellists were asked to score each

210   attribute in reference to the control sample on an unstructured 15 cm line, scaled 0-100, with an

211   anchor defining the average score for the reference product, using Compusense @Hand

212   (Compusense Inc., Guelph, Ontario, Canada). The samples, with three-digit random codes, were

213   introduced in a randomised and balanced order, always with a blind control sample in each session.

214   Between samples, panellists were asked to cleanse their palate with warm water.

215   ***2.7 Statistical Analysis***

216   One-way analysis of variance was performed on the instrumental data to indicate statistical

217   differences between recipes using the Duncan test. ANOVA and Fisher's LSD multiple comparison

218   test at $p = 0.05$ were carried out using the statistical programme Senpaq 5.01 (Qi Statistics Ltd.,

219   Reading, UK).

220   **3. Results and Discussion**

221   ***3.1 Concentration of amino acids and reducing sugars in the potato flake***

222   The potato flake contained relatively low amounts of reducing sugars (6.7±0.24 g/kg glucose and

223   3.7±0.04 g/kg fructose). The concentration of Asn was 2552 mg/kg, similar to previously reported

224   data (Elmore, Koutsidis, Dodson, Mottram, & Wedzicha, 2005). Concentrations of free amino acids

225   in the potato flake were determined (Table S1, see supplementary material) and these values were

226   used to increase their initial concentrations 2 to 100-fold by adding amino acids to the recipe. The

227   amount of added Ala, Leu, Ile, Val and Met was 4 times the naturally occurring amount to make

228   their concentrations 5 times that of the control recipe. However, glycine was one of the lowest

229   amino acids in the potato flake and therefore its concentration was further increased to 100-fold to

230   maintain a level similar to alanine. This corresponded to Gly addition of 0.18% and Ala addition of

231   0.21% (4 times the naturally found level) on potato flake basis. An additional recipe was formed by

232   adding all amino acids (except Asn and γ-aminobutyric acid) at twice the naturally occurring levels.

233   ***3.2 Determination of low thermal input conditions to limit acrylamide formation and monitor***

234   ***alkylpyrazine formation***

235   Apart from the time-temperature conditions used in the main body of this paper (baking at 160 °C

236   for 6 min followed by drying at 130 °C for 10 min), two other conditions, one lower and one higher,

237   were also performed during preliminary trials. At lower temperature conditions (baking at 140 °C

238   for 8 min followed by drying at 110 °C for 15 min), either adding amino acids individually or as binary

239   mixtures did not have a significant effect on alkylpyrazine formation (Figure S1, see supplementary

240   material). Only trimethylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-3-methylpyrazine and 2-ethyl-

241   3,6-dimethylpyrazine were found to be slightly higher when Gly and Ala were added together.

242   Overall, these conditions generated fewer pyrazines than the more severe time-temperature

243   conditions and 2-ethyl-3,5-dimethylpyrazine, one of the key odour-active aroma compounds of

244   interest, was not detected under these conditions at all. The concentration of acrylamide was also

245   very low (10 to 15 ng/g, data not shown). Under these low temperature conditions, the formation

246   of the dicarbonyl/hydroxycarbonyl precursors required for pyrazine formation and acrylamide

247   formation is slow and there were insufficient intermediates formed to significantly increase the

248   concentration of alkylpyrazines. However, the 4-fold addition of Val, Ile, Leu and Met significantly

249 increased the corresponding Strecker aldehydes, by about 4-fold (Figure S1, see supplementary

250 material). It has been previously shown that Strecker aldehydes are kinetically formed earlier than

251 alkylpyrazines in a potato matrix (Low, Mottram, & Elmore, 2006). This may partly explain increases

252 in Strecker aldehydes but no significant changes in alkylpyrazines.

253 At a higher temperature (baking at 160 °C for 6 min followed by drying at 140 °C for 8 min) the

254 concentration of acrylamide in the product was 233±43 ng/g (data not shown), compared to 91±8.2

255 ng/g using the baking conditions discussed in the main body of this article (baking at 160 °C for 6

256 min followed by drying at 130 °C for 10 min). It was possible to observe the effect of amino acid

257 additions on alkylpyrazine formation under both sets of conditions, but only the results (chemical

258 and sensory) for the moderate thermal input conditions are presented below as the focus of the

259 work was on a low acrylamide products.

260 ***3.3 Strecker aldehyde formation and effect of the addition of corresponding amino acids***

261 By adding a targeted mixture of corresponding amino acids (recipe: 4 × Met+Ile+Leu+Val), Strecker

262 aldehydes methional, 2-methylbutanal, 3-methylbutanal, and 2-methylpropanal all increased in

263 similar proportions to the added amounts, namely 499%, 440%, 419% and 437%, respectively

264 (Figure 1). Similarly, addition of Phe at twice the natural level (recipe: all amino acids ×2) increased

265 phenylacetaldehyde to 204%. When the targeted Strecker aldehyde precursor amino acids (×4)

266 were added together with Gly (×100) and Ala (×4), the increases were only 370%, 343%, 318% and

267 326% for methional, 2-methylbutanal, 3-methylbutanal, and 2-methylpropanal , respectively. In

268 accordance with this smaller increase, phenylacetaldehyde decreased 31% in

269 'Gly(×100)+Ala+Met+Ile+Leu+Val' recipe however it was statistically insignificant (p>0.05). Similar

270 decreases were also observed in Gly and Ala added samples with respect to control however they

271 were also not significant. This smaller increase in the presence of Gly and Ala is likely to be due to

272 the fact that amino acids compete with each other for the available dicarbonyls, increasing the

273 Strecker aldehydes derived from Gly and Ala (formaldehyde and acetaldehyde) whilst decreasing

274 the others. This observation was also evident in a previous study in which 1.3% Gly on dry basis was

275 added to a potato model system cooked at 180 °C (Low et el, 2006).

276 In addition to changes in Strecker aldehydes, dimethyl disulfide and dimethyl trisulfide, the

277 degradation products of methionine and methional, increased 98% and 41% in the

278 'Met+Ile+Leu+Val' recipe, 139% and 148% in 'Gly(×100)+Ala+Met+Ile+Leu+Val' recipe and 45% and

279 43% in 'all amino acids' recipe, respectively (Table S2, see supplementary material). These sulfide

280 compounds have very low odour thresholds and they also contribute to the cooked flavour in

281 potato-based matrices (Martin & Ames, 2001).

282 **3.4 Effect of amino acid addition on alkylpyrazine formation**

283 Addition of individual amino acids Gly, Ala, Gln and also Ser+Thr was performed at the higher

284 thermal input condition mentioned above, and there were no changes in alkylpyrazines (Figure S2,

285 see supplementary material). A binary mixture of Ser and Thr without carbonyls has been shown to

286 produce certain alkylpyrazines under pyrolytic conditions (Shu, 1999), and Gln, Ser and Thr have

287 been shown to produce various alkylpyrazines in the presence of reducing sugar at pH 8 and 160 °C

288 (Chen & Ho, 1999). However, these conditions do not correspond to the low thermal input

289 conditions used here. On the other hand, when Gly and Ala were added together significant

290 increases in certain alkylpyrazines were observed at both thermal input conditions (Figure 1; and

291 Figure S2, see supplementary material).

292 Pyrazine, methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, ethylpyrazine, 2,3-

293 dimethylpyrazine and 2-ethyl-5-methylpyrazine did not change considerably with amino acid

294 addition with respect to control (Table S2, see supplementary material). As shown in Figure 1,

295 trimethylpyrazine increased by 81% and 134% when Gly was increased 100 fold in the recipes

296 Gly(×100)+Ala and Gly(×100)+Ala+Met+Ile+Leu+Val, respectively ($p<0.05$). In Gly(×20)+Ala recipe,

297 the increase in trimethylpyrazine was 44% without statistical significance ($p>0.05$). This is consistent

298 with the non-oxidative route for formation of pyrazines, where one of the methyl substituents

299 comes from Gly, probably via formaldehyde.

300 Remarkable increases were observed for 2-ethyl-6-methylpyrazine, 2-ethyl-3-methylpyrazine, 2,6-

301 diethylpyrazine, 2,3-diethylpyrazine, and more importantly for the lowest odour threshold

302 trisubstituted pyrazines 2-ethyl-3,6-dimethylpyrazine and 2-ethyl-3,5-dimethylpyrazine, when Gly

303 and Ala were added together. The amounts were more than twice or three times that of the control

304 recipe in most cases with statistical significance. The increase in 2-ethyl-3,6-dimethylpyrazine was

305 261%, 210% and 348% in 'Gly(×20)+Ala', 'Gly(×100)+Ala' and 'Gly(×100)+Ala+Met+Ile+Leu+Val'

306 recipes, respectively ($p<0.05$). 2-Ethyl-3,5-dimethylpyrazine increased 98% ($p>0.05$), 142% and

307 155% ($p<0.05$) in 'Gly(×20)+Ala', 'Gly(×100)+Ala' and 'Gly(×100)+Ala+Met+Ile+Leu+Val' recipes,

308 respectively. These trisubstituted pyrazine isomers 2-ethyl-3,6-dimethylpyrazine and 2-ethyl-3,5-

309 dimethylpyrazine have odour thresholds (3.6 ng/L air and 0.011 ng/L air respectively), particularly

310 compared to other pyrazines (Table S2) and have been shown to contribute to the baked flavour in

311 a potato matrix (Wagner & Grosch, 1998).

312 Addition of all free amino acids (except Asn and GABA) at twice that of the natural level did not have

313 a significant effect on the alkylpyrazine formation, neither did addition of Gly and Ala separately

314 (see above). However, Figure 1 shows that addition of Gly and Ala together increased the formation

315 of key alkylpyrazines with low odour thresholds in low acrylamide cooked potato-based matrices,

316 whereas the addition of Gly alone did not have this effect, at least in the high temperature baked

317 products. However, Low et al (2006) showed an increase (>100%) in a wider range of pyrazines

318 (particularly 2,3-dimethylpyrazine, trimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, and

319   tetramethylpyrazine) when Gly (1.3% on dry basis) was added to mitigate the formation of

320   acrylamide in potato cakes baked at 180 °C. The reason for not observing the effect of individual

321   addition of Gly on alkylpyrazine formation in this study might be due to the low thermal input

322   condition used to prepare low acrylamide potato products, and also the added Gly was 7.2-fold

323   lower.

324   Both Gly and Ala have many possible roles in the Maillard reaction. They participate in the first step

325   of the Maillard reaction and facilitate the formation of α-dicarbonyl compounds from reducing

326   sugars which are important precursors of pyrazines. However, they can also compete with the other

327   amino acids for Strecker degradation of the available carbonyls. Additionally, Strecker degradation

328   of Gly and Ala yield their Strecker aldehydes (formaldehyde and acetaldehyde respectively) and α-

329   amino carbonyl compounds, both of which are intermediates in alkylpyrazine formation.

330   Formaldehyde and acetaldehyde formed from Gly and Ala also participate in the chain elongation

331   process of sugar-derived α-dicarbonyl compounds and these α-dicarbonyl compounds with ethyl

332   and/or methyl substituents from amino acids may be involved in alkylpyrazine formation through

333   oxidative or non-oxidative pathways. In the oxidative pathway, the dihydroalkylpyrazine

334   intermediates readily oxidise to form the corresponding alkylpyrazines and this has been shown to

335   be a major pathway (Chu & Yaylayan, 2009). Formaldehyde and acetaldehyde formed from Gly and

336   Ala participate in the non-oxidative pathway of pyrazine formation and yield ethyl or methyl

337   substituted alkylpyrazines upon dehydration of the alkylated site of dihydropyrazines.

338   It is interesting that the 2-ethyl-3,6-dimethylpyrazine is influenced more than 2-ethyl-3,5-

339   dimethylpyrazine by the addition of alanine, suggesting greater incorporation of the alanine

340   backbone into the molecule via the non-oxidative mechanism. The 2-ethyl-3,6-dimethylpyrazine is

341   also formed in greater quantities than the 2-ethyl-3,5-dimethylpyrazine, which was not detected at

342   all under the lower temperature conditions. The 2-ethyl-3,6-dimethylpyrazine can be formed by the

343 condensation of two molecules of 1-amino-2-propanone (from methylglyoxal, and sterically

344 favoured over 2-aminopropanal) and incorporation of acetaldehyde (from alanine) (Figure S3). The

345 former is readily formed and more reactive than higher molecular weight dicarbonyls (Hofmann,

346 1999) such as 2,3-pentanedione which is required for the oxidative pathway. This may explain both

347 the greater quantity formed and the greater response to alanine addition. Although it is hard to

348 clarify the mechanism, it is clear that Gly and Ala are effective together in increasing certain

349 pyrazines, especially the lower threshold trisubstituted isomers, in low acrylamide cooked potato

350 model systems.

351 ***3.5 Targeted addition of carbonyl compounds as carbon backbones for alkylpyrazine formation***

352 The carbonyl pool formed in the intermediate stage of Maillard reaction can also be manipulated

353 by adding 2,3-pentanedione (PD) and 3,4-hexanedione (HD) to provide the required carbon

354 backbone for alkylpyrazine formation. They can be converted into their corresponding α-

355 aminocarbonyl derivatives which may then participate in the alkylpyrazine formation in the

356 presence of amino compounds. Addition of 100 mg/kg 2,3-pentanedione on a dry basis increased

357 its headspace concentration 15 and 10 times in cooked 2,3-PD and 2,3-PD-Leu-Ile-Met-Val+Phe

358 recipes, respectively. There was no 3,4-hexanedione in the headspace of the control sample and its

359 addition level was 100 mg/kg dry basis as well.

360 Addition of 2,3-pentanedione alone increased 2-ethyl-3-methylpyrazine and 2-ethyl-3,5-

361 dimethylpyrazine 155% and 121% with respect to control recipe (Figure 2) ($p < 0.05$). On the other

362 hand, 2-ethyl-3,6-dimethylpyrazine did not change when 2,3-pentanedione added alone. We

363 speculate that this is because the methyl-substituted second carbon in 2,3-pentanedione is less

364 sterically hindered than the ethyl substituted third carbon and it is preferably transformed into 2-

365 amino-3-pentanone during Strecker degradation. 2-Amino-3-pentanone condenses with 1-amino-

366 2-propanone, which for the same reason is preferably formed from methylglyoxal instead of 2-

367 aminopropanal, and produces 2-ethyl-3,5-dimethylpyrazine (See supplementary material for the

368 proposed mechanism, Figure S3). Therefore, the addition of 2,3-pentanedione targets the formation

369 of lower odour threshold trisubstituted isomer 2-ethyl-3,5-dimethylpyrazine in the potato matrix.

370 Adding Leu, Ile, Met, Val and Phe together with 2,3-pentanedione increased 2-ethyl-3-

371 methylpyrazine, 2-ethyl-3,6-dimethylpyrazine and 2-ethyl-3,5-dimethylpyrazine even more (272%,

372 146% and 390%, respectively), as more amino acids aided the transformation of α-dicarbonyls into

373 their α-aminocarbonyl derivatives during the Strecker degradation ($p<0.05$). Scalone et al (2019)

374 also suggested this pathway of 2-ethyl-3,5-dimethylpyrazine formation from 2,3-pentanedione in

375 whey protein hydrolysate-glucose model systems (pH 7.8 phosphate buffer) heated at 180 C for 90

376 min.

377 Addition of 3,4-hexanedione alone increased 2,3-diethylpyrazine by 84% however, it was not

378 statistically different to the control recipe (Figure 2). A further increase in 2,3-diethylpyrazine

379 (338%) was achieved when Leu, Ile, Met, Val and Phe were also added ($p<0.05$). 2,3-Diethyl-5-

380 methylpyrazine was not detected in the control sample in this set of baking. By the addition of 3,4-

381 hexanedione alone and together with Leu, Ile, Met, Val and Phe, 2,3-diethyl-5-methylpyrazine was

382 detected at levels comparable to 2-ethyl-3,5-dimethylpyrazine. This pyrazine has a roast, potato

383 aroma and a low threshold, and is known to be important in baked potato aroma.

384 *3.6 Effect of whey protein hydrolysate addition on alkylpyrazine formation*

385 Addition of whey protein hydrolysate to enhance the Maillard reaction and also as an amine source

386 when it is added together with 2,3-pentanedione and 3,4-hexanedione was assessed for its effect

387 on alkylpyrazine formation. The total concentration of free amino acids in whey protein hydrolysate

388 was 75 mg/kg and they did not contribute to the free amino acid pool in potato flake, which was

389 1600-fold higher. Therefore, there were no changes for Strecker aldehydes (Table S3, see

390 supplementary material). On the other hand, all alkylpyrazines increased considerably by the

391 addition of 5% whey protein hydrolysate on dry basis (Figure 3). The differences for some

392 alkylpyrazines were statistically insignificant due to the higher baking variance between replicates

393 in this set of baking although the concentrations were more than doubled with respect to control.

394 Interestingly, a minor increase (30%) was observed only for unsubstituted pyrazine while others

395 increased at least one-fold or two (Table S2, see supplementary material). Pyrazine is formed from

396 two molecules of glyoxal, and vinylic pyrazines were also increased by the addition of whey protein

397 hydrolysate. These changes indicated that whey protein hydrolysate increases overall Maillard

398 reaction and thus aiding alkylpyrazine formation by enhancing the carbonyl pool at the intermediate

399 stage. The pH of the dough was 5.92 ± 0.01 and it did not change by the addition of 5% whey protein

400 hydrolysate (5.92 ± 0.01). It has been shown that the N-terminal amino groups found in peptides are

401 also effective for the formation of pyrazines with a different mechanism that is observed in the

402 Strecker degradation of free amino acids (Van Lancker et al, 2010, 2012). Scaleno et al (2015)

403 showed that whey protein hydrolysate contributes to the formation of pyrazines significantly more

404 than free amino acids during heating whey protein hydrolysate and glucose mixture with phosphate

405 buffer at ph 7.8. Moreover, by addition of 2,3-pentanedione and 3,4-hexanedione together with

406 whey protein hydrolysate, considerably higher amounts of corresponding alkylpyrazines were

407 observed (Figure 3).

408 ***3.7 Changes in acrylamide formation by recipe modifications***

409 The concentration of acrylamide in the control recipe was 91 ± 8.2 ng/g and it did not change by the

410 addition of mixtures of amino acids except 'all amino acids $\times 2$' recipe (Figure 4A) ($p > 0.05$). Adding

411 all amino acids at twice the level of those naturally present, except asparagine and γ-aminobutyric

412 acid, decreased acrylamide formation by 42% ($p < 0.05$). The initial ratio of asparagine to total amino

413   acids was 34% and decreased to 16% when all amino acids (except asparagine and γ-aminobutyric

414   acid) were 3 times their initial level.

415   In a different set of baking, the acrylamide concentration of the control sample was 78±2.2 ng/g

416   (Figure 4B). Addition of 100 mg/kg 2,3-pentanedione and 5% whey protein hydrolysate (both on dry

417   basis) did not change the formation of acrylamide with respect to control ($p>0.05$). On the other

418   hand, a 33% increase in acrylamide was observed when 3,4-hexanedione (100 mg/kg on dry basis)

419   was added alone ($p<0.05$) and this effect was not apparent when it was added together with amino

420   acids. The increase in acrylamide formation by addition of 3,4-hexanedione could stem from the

421   presence of 4-hydroxy-3-hexanone in the aroma as an impurity which was identified in GC-MS

422   chromatogram. It has been shown that α-hydroxy functionality in carbonyl compounds favours

423   acrylamide formation (Stadler, Robert, Riediker, Varga, Davidek, Devaud, et al., 2004).

424   **3.8 *Colour***

425   The addition of free amino acids or α-dicarbonyl compounds did not change surface browning. On

426   the other hand, in samples where whey protein hydrolysate had been added there was more surface

427   browning and sensory analysis was performed under red light.

428   ***3.9 Sensory Analysis of Selected Recipes***

429   Quantitative descriptive sensory evaluation of selected amino acid added samples showed that

430   increasing alkylpyrazines (Gly(×100)+Ala) and Strecker aldehydes (Leu+Ile+Val+Met) separately did

431   not have any effect on baked aroma and flavour (Table 1) ($p>0.05$). Increasing alkylpyrazines and

432   Strecker aldehydes together, as in Gly(×100)+Ala+Leu+Ile+Val+Met, was found to be necessary for

433   an increase in the perception of baked aroma and flavour. This was the case for overall aroma

434   intensity and overall flavour intensity as well. There were no changes for other attributes.

435   Interestingly, no significant increase in boiled potato aroma and flavour was observed even though

436   methional was increased considerably. On the other hand, a significant increase for boiled potato

437   aroma was determined in the samples with methionine baked at lower temperature conditions

438   when there was no difference for baked aroma and flavour according to the quantitative descriptive

439   sensory evaluation (data not shown).

440   As mentioned above, the addition of 2,3-pentanedione alone increased the lowest threshold

441   alkylpyrazine 2-ethyl-3,5-dimethylpyrazine by 121% with respect to the control recipe, however, it

442   had no effect on baked aroma and flavour (Table 2). Baked flavour, baked aroma and baked

443   aftertaste increased when 2,3-pentanedione and the Strecker aldehyde precursor amino acids were

444   added together. 3,4-Hexanedione addition together with Leu+Ile+Val+Met+Phe increased baked

445   aroma but not baked flavour. It should be noted that in this set of baking phenylalanine was also

446   added in Strecker mix of amino acids different from the samples given in the previous sensory

447   analysis. As phenylacetaldehyde has a floral and honey like flavour, it changed the flavour profile

448   and panellist were agreed on the presence of a cheesy attribute in certain samples during the

449   training session of this set of samples and it was added to the vocabulary list. Quantitative

450   descriptive sensory evaluation indicated that cheesy attribute appeared when there is

451   phenylalanine in the recipe. Only the control and the sample containing just 2,3-pentanedione had

452   a score of zero for cheesy. It should be mentioned that no buttery aroma and flavour was described

453   by the panellists in 2,3-pentanedione added samples during training sessions and this was later

454   assessed by introducing butter and also food grade 2,3-pentanedione aroma solution to the

455   panellists. Similarly, panellist did not describe 3,4-hexanedione aroma and flavour (caramel, nutty,

456   buttery) in these samples as confirmed also by introducing its food grade solution to the panellist.

457   In all whey protein hydrolysate added samples, overall aroma intensity was found to be higher but

458   there were no differences for baked aroma and flavour even though there were considerable

459   increases in alkylpyrazines (sensory data not shown). As mentioned above, there were no increases

460   in Strecker aldehydes in these samples. Therefore, increasing only alkylpyrazines did not contribute

461   to the perception of baked aroma and flavour when there were no increments of Strecker

462   aldehydes.

463   **4. Conclusion**

464   This work shows that it is possible to enhance baked flavour in low acrylamide potato-based

465   products (~80 ng/g acrylamide), by adding a specifically targeted combination of amino acids and

466   key intermediates, without increasing acrylamide concentration. Adding the full complement of free

467   amino acids (excluding asparagine) did not have the same effect, nor did addition of hydrolysed

468   whey protein. The best combination was glycine, alanine, valine, leucine, isoleucine and methionine,

469   a combination which was required to boost both Strecker aldehydes and pyrazines. The data add to

470   our understanding of the role of precursor mixtures and highlights merit for a targeted approach

471   over a single source of protein. Appropriate selection of food ingredients to reflect the desired

472   amino acid profile would be required. Similar chemistry has been demonstrated in binary or tertiary

473   aqueous model systems, but one of the achievements of this paper is to show how this can be

474   applied in a complex food matrix where up to 6 amino acids were used to achieve the desired effect.

475

476   **Declaration**

480   **References**

481   Amrani-Hemaimi, M., Cerny, C., & Fay, L. B. (1995). Mechanisms of formation of alkylpyrazines in

482   the   Maillard   reaction.   *J.   Agric.   Food   Chem.,   43*(11),   2818-2822.

483   https://doi.org/10.1021/jf00059a009

484   Buttery, R. G., Seifert, R. M., Guadagni, D. G., & Ling, L. C., (1971). Characterization of volatile

485   pyrazine and pyridine components of potato chips. *J. Agric. Food Chem., 19*(5), 969-71.

486   Cerny, C., & Grosch, W. (1994). Precursors of ethyldimethylpyrazine isomers and 2,3-diethyl-5-

487   methylpyrazine   formed   in   roasted   beef.   *Z.   Lebensm.   Unters.   Forsch.,   198*(3),   210-214.

488   https://doi.org/10.1007/BF01192597

489   Chen, J. H., & Ho, C. T. (1999). Comparison of volatile generation in serine/threonine/glutamine-

490   ribose/glucose/fructose   model   systems.   *J.   Agric.   Food   Chem.,   47*(2),   643-647.

491   https://doi.org/10.1021/jf980771a

492   Chu, F. L., & Yaylayan, V. A. (2009). Isotope labeling studies on the origin of 3,4-hexanedione and

493   1,2-butanedione in an alanine/glucose model system. *J. Agric. Food Chem., 57*(20), 9740-9746.

494   https://doi.org/10.1021/jf902117v

495   Elmore, J. S., Koutsidis, G., Dodson, A. T., Mottram, D. S., & Wedzicha, B. L. (2005). Measurement of

496   acrylamide and its precursors in potato, wheat, and rye model systems. *J. Agric. Food Chem., 53*(4),

497   1286-1293. https://doi.org/10.1021/jf048557b

498   Gokmen, V., Acar, O. C., Koksel, H., & Acar, J. (2007). Effects of dough formula and baking conditions

499   on acrylamide and hydroxymethylfurfural formation in cookies. *Food Chem., 104*(3), 1136-1142.

500   https://doi.org/10.1016/j.foodchem.2007.01.008

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

502 of pentoses and hexoses with L-alanine. *Eur. Food Res. Technol.,* (1999) *209*, 113–121.

503 https://doi.org/10.1007/s002170050468

504 International Agency for Research on Cancer. (1994). *IARC Monographs on the Evaluation of*

505 *Carcinogenic Risks to Humans 60*. Lyon, France: International Agency for Research on Cancer (IARC).

506 Kocadağlı, T. Özdemir, K.S., Gökmen, V. (2013). Effects of infusion conditions and decaffeination on

507 free amino acid profiles of green and black tea. *Food Res. Int. 53*(2), 720-725.

508 Low, M. Y., Koutsidis, G., Parker, J. K., Elmore, J. S., Dodson, A. T., & Mottram, D. S. (2006). Effect of

509 citric acid and glycine addition on acrylamide and flavor in a potato model system. *J. Agric. Food*

510 *Chem., 54*(16), 5976-5983. https://doi.org/10.1021/jf060328x

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

512 the generation of flavour in heated foods. In W. L. P. Bredie & M. A. Petersen (Eds.), *Developments*

513 *in Food Science*, vol. 43 (pp. 363-366): Elsevier.

514 Martin, F. L., & Ames, J. M. (2001). Formation of Strecker aldehydes and pyrazines in a fried potato

515 model system. *J. Agric. Food Chem., 49*(8), 3885-3892. https://doi.org/10.1021/jf010310g

516 Mottram, D. S., Wedzicha, B. L., & Dodson, A. T. (2002). Acrylamide is formed in the Maillard

517 reaction. *Nature, 419*(6906), 448-449. https://doi.org/10.1038/419448a

518 Palermo, M., Gökmen, V., De Meulenaer, B., Ciesarová, Z., Zhang, Y., Pedreschi, F., & Fogliano, V.

519 (2016). Acrylamide mitigation strategies: critical appraisal of the FoodDrinkEurope toolbox. *Food*

520 *and Funct., 7*(6), 2516-2525. https://doi.org/10.1039/c5fo00655d

521 Roberts, A. (2016). The Safety and Regulatory Process for Amino Acids in Europe and the United

522 States. *J. Nutr. 146*(12), 2635S-2642S. https://doi.org/10.3945/jn.116.234591

523   Scalone, G.L.L., Cucu, T., De Kimpe, N., De Meulenaer, B., (2015). Influence of Free Amino Acids,

524   Oligopeptides, and Polypeptides on the Formation of Pyrazines in Maillard Model Systems. *J. Agric.*

525   *Food Chem., 63,* 5364-5372. https://doi.org/10.1021/acs.jafc.5b01129

526   Scalone, G.L.L., Lamichhane, P., Cucu, T., De Kimpe, N., De Meulenaer, B., (2019). Impact of different

527   enzymatic hydrolysates of whey protein on the formation of pyrazines in Maillard model systems.

528   *Food Chem., 278,* 533-544. https://doi.org/10.1016/j.foodchem.2018.11.088

529   Shibamoto, T., & Bernhard, R. A. (1977). Investigation of pyrazine formation pathways in glucose-

530   ammonia   model   systems.   *Agric.   Biol.   Chem.,   41*(1),   143-153.

531   https://doi.org/10.1080/00021369.1977.10862443

532   Shu, C. K. (1999). Pyrazine formation from serine and threonine *J. Agric. Food Chem., 47*(10), 4332-

533   4335. https://doi.org/10.1021/jf9813687

534   Stadler, R. H., Blank, I., Varga, N., Robert, F., Hau, J., Guy, P. A., Robert, M. C., & Riediker, S. (2002).

535   Acrylamide   from   Maillard   reaction   products.   *Nature,   419*(6906),   449-450.

536   https://doi.org/10.1038/419449a

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

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

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

540   https://doi.org/10.1021/jf0495486

541   Taeymans, D., Wood, J., Ashby, P., Blank, I., Studer, A., Stadler, R. H., Gonde, P., Van Eijck, P., Lalljie,

542   S., Lingnert, H., Lindblom, M., Matissek, R., Muller, D., Tallmadge, D., O'Brien, J., Thompson, S.,

543   Silvani, D., & Whitmore, T. (2004). A review of acrylamide: an industry perspective on research,

544   analysis, formation and control. Crit. Rev. Food Sci.*, 44*(5), 323-347.

545   Van Lancker, F., Adams, A., & De Kimpe, N. (2010). Formation of pyrazines in Maillard model systems

546   of lysine-containing dipeptides. *J. Agric. Food Chem., 58*(4), 2470-2478.

547   https://doi.org/10.1021/jf903898t

548   Van Lancker, F., Adams, A., & De Kimpe, N. (2012). Impact of the N-terminal amino acid on the

549   formation of pyrazines from peptides in Maillard model systems. *J. Agric. Food Chem., 60*(18), 4697-

550   4708. https://doi.org/10.1021/jf301315b

551   Wagner, R., Czerny, M., Bielohradsky, J., & Grosch, W. (1999). Structure odour-activity relationships

552   of alkylpyrazines. *Z. Lebensm. Unters. Forsch., 208*(5-6), 308-316.

553   https://doi.org/10.1007/s002170050422

554   Wagner, R. K., & Grosch, W. (1998). Key odorants of French fries. *J. Am. Oil Chem. Soc., 75*(10), 1385-

555   1392. https://doi.org/10.1007/s11746-998-0187-4

556   Yaylayan, V. A. (1997). Classification of the Maillard reaction: a conceptual approach. *Trends Food*

557   *Sci. Technol., 8*(1), 13-18. https://doi.org/10.1016/S0924-2244(96)20013-5

558   Yaylayan, V. A. (2003). Recent advances in the chemistry of Strecker degradation and Amadori

559   rearrangement: Implications to aroma and color formation. Food Sci. Technol. Res.*, 9*(1), 1-6.

560   https://doi.org/10.3136/fstr.9.1

561 **Figure Captions**

562 **Figure 1.** Relative amounts of Strecker aldehydes and selected alkylpyrazines formed in a potato-

563 based matrix with or without the addition of amino acids. Bars with the same lowercase letters

564 above the bars indicate no statistically significant difference between them (p>0.05).

565 **Figure 2.** Effect of 2,3-pentanedione (2,3-PD) and 3,4-hexanedione (3,4-HD) addition on the

566 corresponding alkylpyrazines. Bars with the same lowercase letters above the bars indicate no

567 statistically significant difference between them (p>0.05).

568 **Figure 3.** Effect of whey protein hydrolysate (WPH) addition on alkylpyrazine formation also

569 together with 2,3-pentanedione (2,3-PD) and 3,4-hexanedione (3,4-HD). Bars with the same

570 lowercase letters above the bars indicate no statistically significant difference between them

571 (p>0.05).

572 **Figure 4.** The concentration of acrylamide formed in different recipes. Samples in A and B graphs

573 are different sets of baking and they have their own control sample. * indicates a statistically

574 significant difference within a group (p≤0.05).

Table 1. Quantitative descriptive sensory evaluation of selected amino acid added samples

	Control	Gly(×100)+Ala	Leu+Ile+Val+Met	Gly(×100)+Ala+Leu+Ile+Val+Met	ANOVA p-value
Aroma					
Overall aroma intensity	38^c	38^c	39^{bc}	44^a	0.01
Baked	38^b	36^b	40^{ab}	43^a	0.02
Boiled potato	5	5	6	5	0.74
Fatty	24	23	22	24	0.19
Sweet	13	13	15	16	0.13
Taste					
Sweet	17	16	13	16	0.07
Salty	14	16	15	16	0.26
Bitter	3	1	4	3	0.22
Umami	18	19	17	21	0.24
Flavour					
Overall flavour intensity	38^c	39^c	40^{bc}	45^{ab}	0.02
Baked	39^b	38^b	39^b	44^a	0.01
Boiled potato	6	7	7	7	0.85
Fatty	24	21	20	20	0.08
Aftertaste					
Baked	34	34	34	38	0.07
Boiled potato	4	5	6	5	0.50
Sweet	17	16	15	18	0.15
Salty	15	15	15	16	0.04
Umami	17	17	16	19	0.08
Fatty	18	15	15	16	0.13

Mean scores (n=12) Within a row, scores with the same lowercase letters are not statistically different (p>0.05).

Table 2. Quantitative descriptive sensory evaluation of 2,3-pentanedione and 3,4-hexanedione added samples

	Control	2,3-PD	2,3-PD+Leu+Ile +Val+Met+Phe	3,4-HD+Leu+Ile +Val+Met+Phe	Leu+Ile+Val +Met+Phe	ANOVA p-value
Aroma						
Overall aroma intensity	33.5^b	29.4^c	40.3^a	36.1^b	36.6^{ab}	0.0001
Baked	35.0^b	33.2^b	42.0^a	39.6^a	40.4^a	0.0015
Boiled potato	5.5	4.7	4.3	4.4	4.3	0.8745
Fatty	17.1^{bc}	15.3^c	21.1^a	21.3^a	19.5^{ab}	0.0011
Sweet	12.1	11.8	12.7	12.4	12.0	0.9731
Cheesy	0.0^b	0.0^b	5.9^a	1.6^b	3.6^{ab}	0.0293
Taste						
Sweet	13.2	12.0	12.7	11.8	11.3	0.6764
Salty	13.6	12.5	13.5	13.4	12.8	0.8655
Bitter	2.5^b	2.6^b	6.8^a	6.2^a	2.0^b	0.0011
Umami	12.4^{bc}	11.1^c	15.8^a	13.9^{abc}	14.8^{ab}	0.0366
Flavour						
Overall flavour intensity	32.3^b	26.7^c	43.4^a	32.9^b	35.2^b	<.0001
Baked	32.3^b	24.3^c	39.7^a	33.3^b	35.5^{ab}	<.0001
Boiled potato	6.0	7.3	5.1	4.4	3.1	0.2763
Fatty	11.2^{bc}	10.9^c	15.9^a	14.0^{ab}	14.1^a	0.0064
Cheesy	0.0^b	0.0^b	10.6^a	0.8^b	4.0^b	0.0001
Aftertaste						
Baked	31.0^b	23.1^c	38.4^a	31.7^b	33.8^{ab}	0.0003
Boiled potato	5.1^{ab}	5.8^a	3.2^b	3.9^{ab}	3.1^b	0.1390
Sweet	8.6	7.7	9.1	8.0	8.6	0.6608
Salty	11.3	9.3	12.2	10.9	11.5	0.0705
Umami	10.9^{bc}	9.0^c	13.9^a	11.9^{ab}	12.7^{ab}	0.0127
Fatty	10.4^{bc}	9.2^c	13.7^a	12.8^{ab}	13.1^{ab}	0.0177
Cheesy	0.0^b	0.0^b	6.3^a	0.5^b	2.6^{ab}	0.0261

Mean scores (n=12) Within a row, scores with the same lowercase letters are not statistically different (p>0.05).
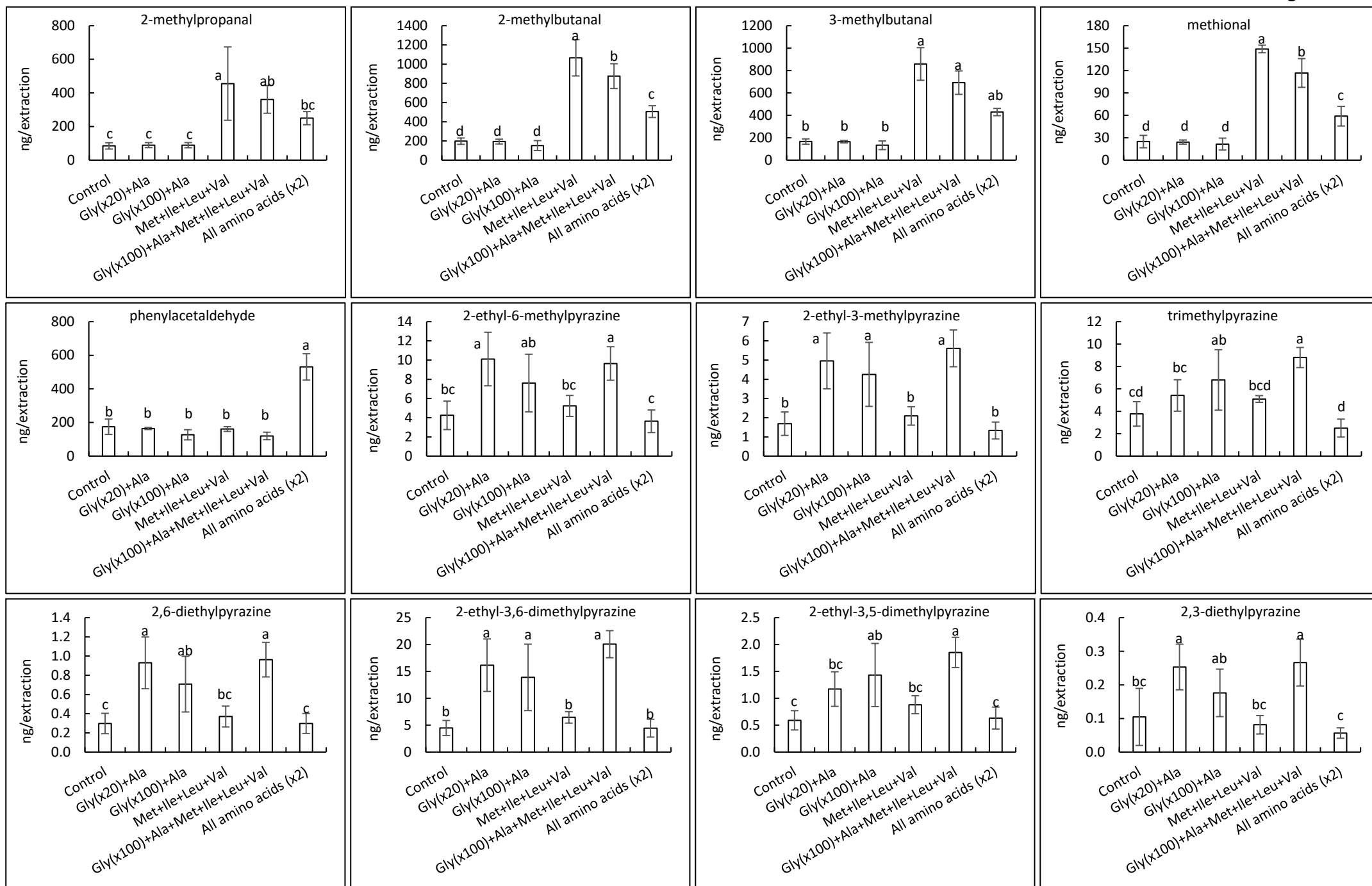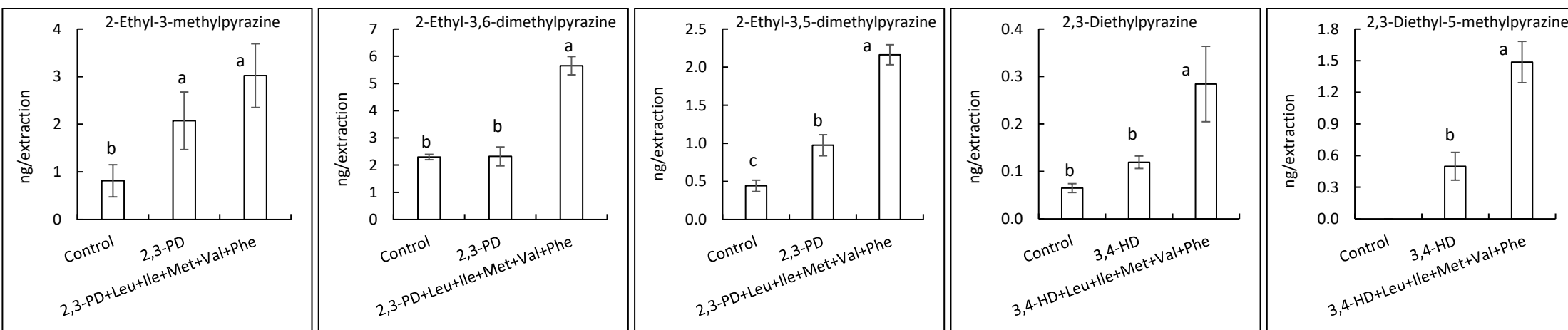
Figure 1

Figure 2

Figure 2

Figure 3

Figure 4
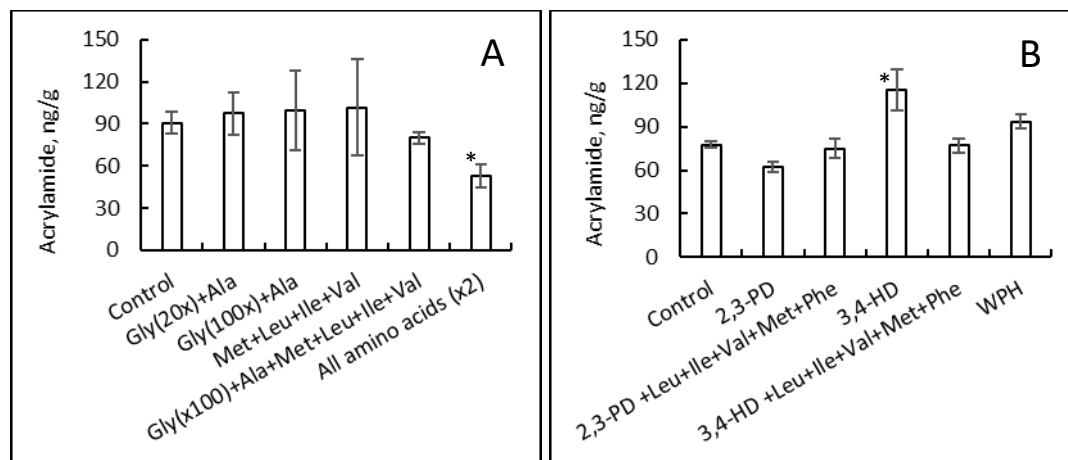
Figure S1. Formation of Strecker aldehydes and selected alkylpyrazines by addition of individual amino acids and also binary mixtures of amino acids during baking at 140 ˚C for 8 min followed by drying at 110 ˚C for 15 min (unless indicated amounts of added amino acids are 4-times the naturally occurring levels)
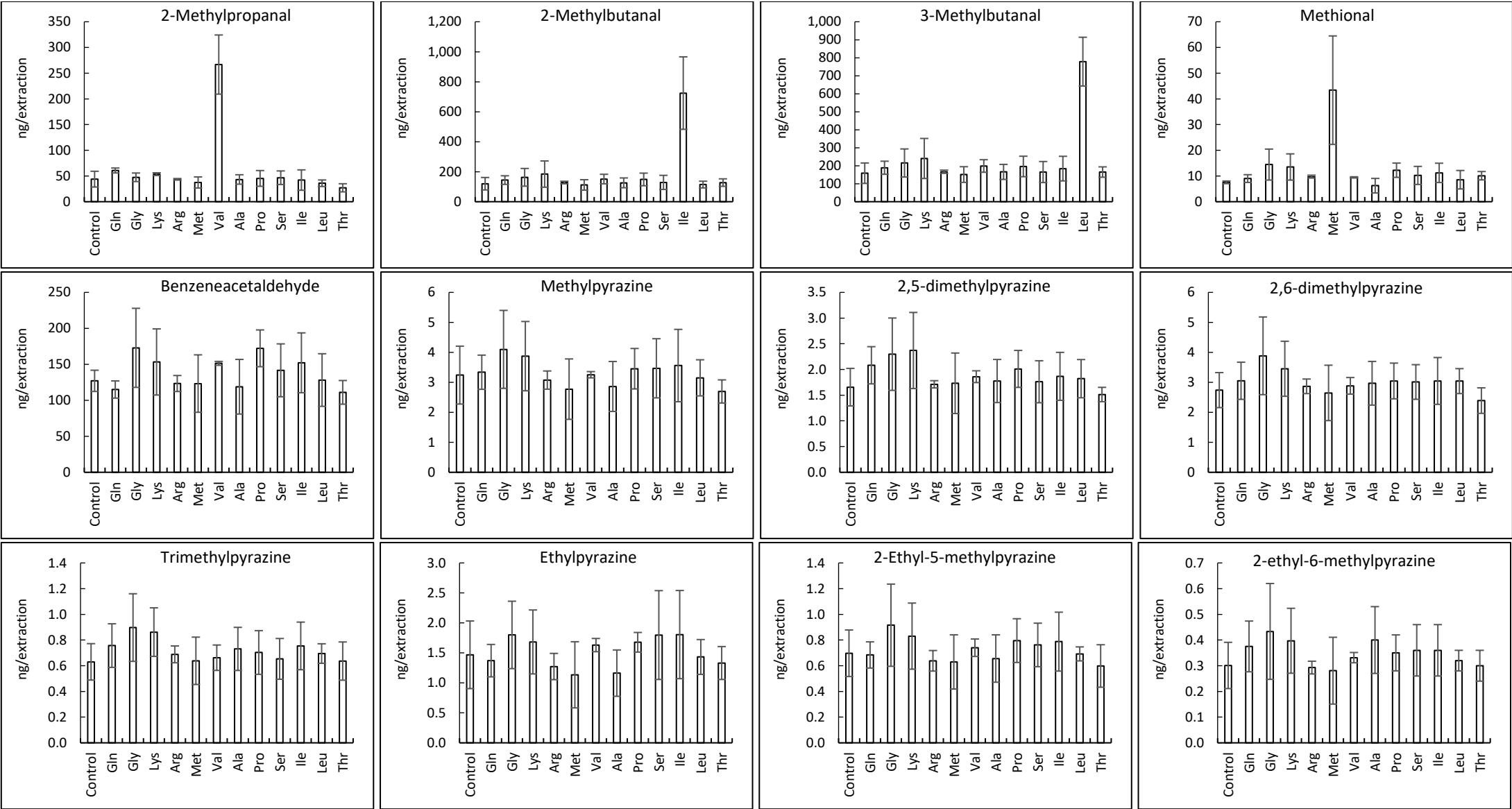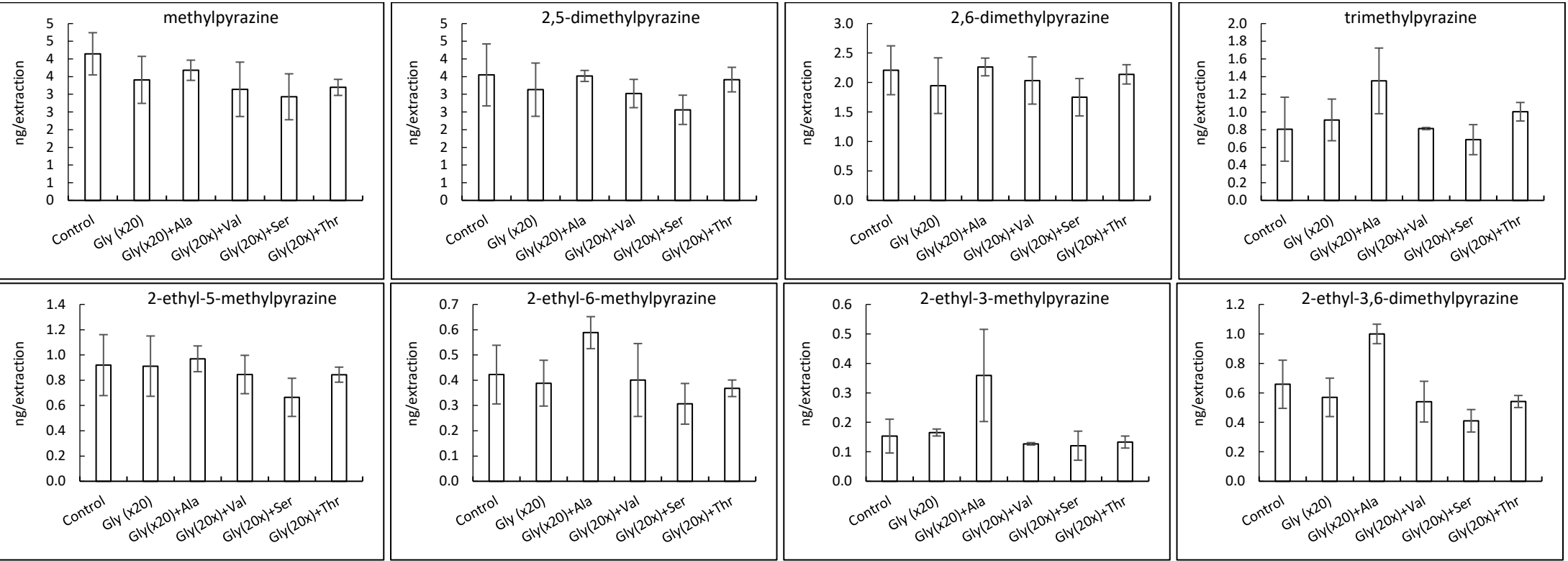
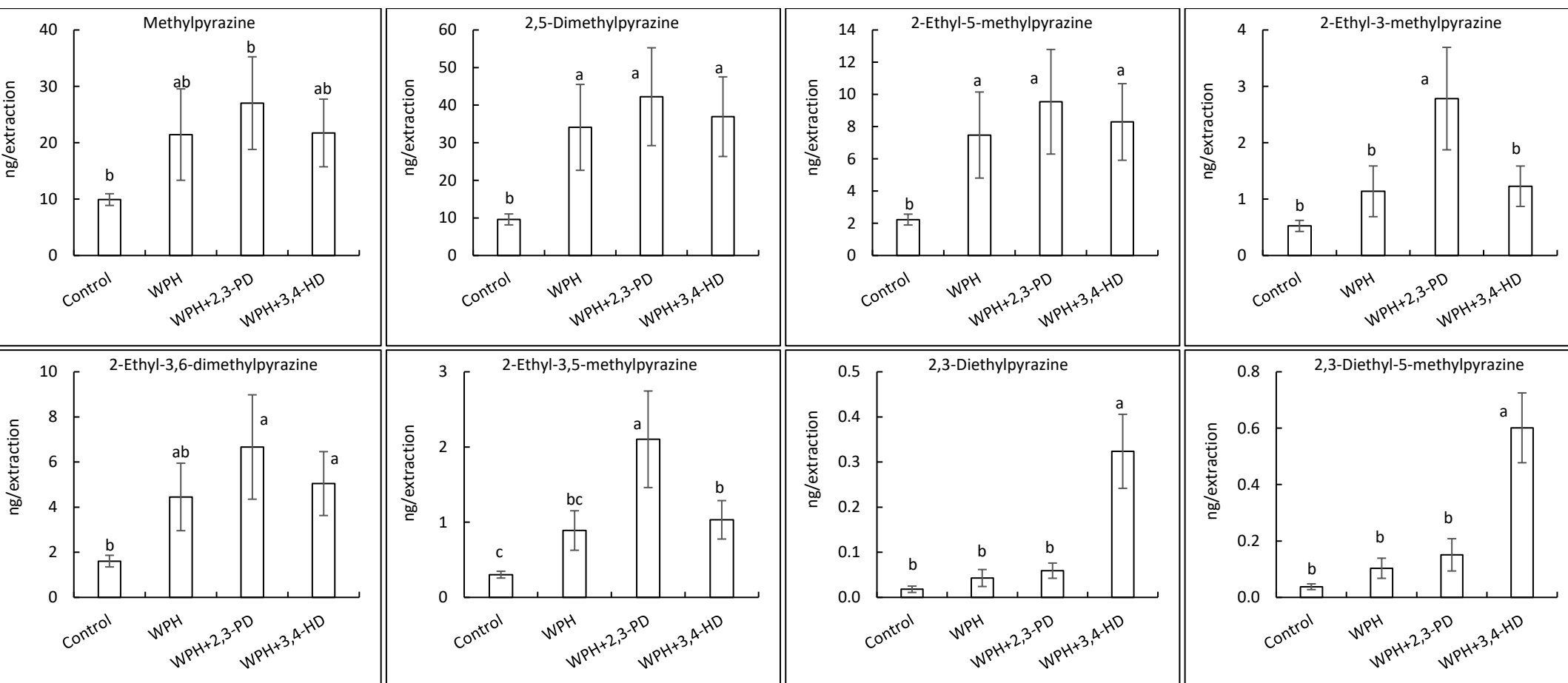**Figure S2.** Formation of selected alkylpyrazines by addition of individual amino acids and also binary mixtures of amino acids during baking at 160 ˚C for 6 min followed by drying at 140 ˚C for 8 min

(Unless indicated amounts of added amino acids are 4-times the naturally occurring levels)
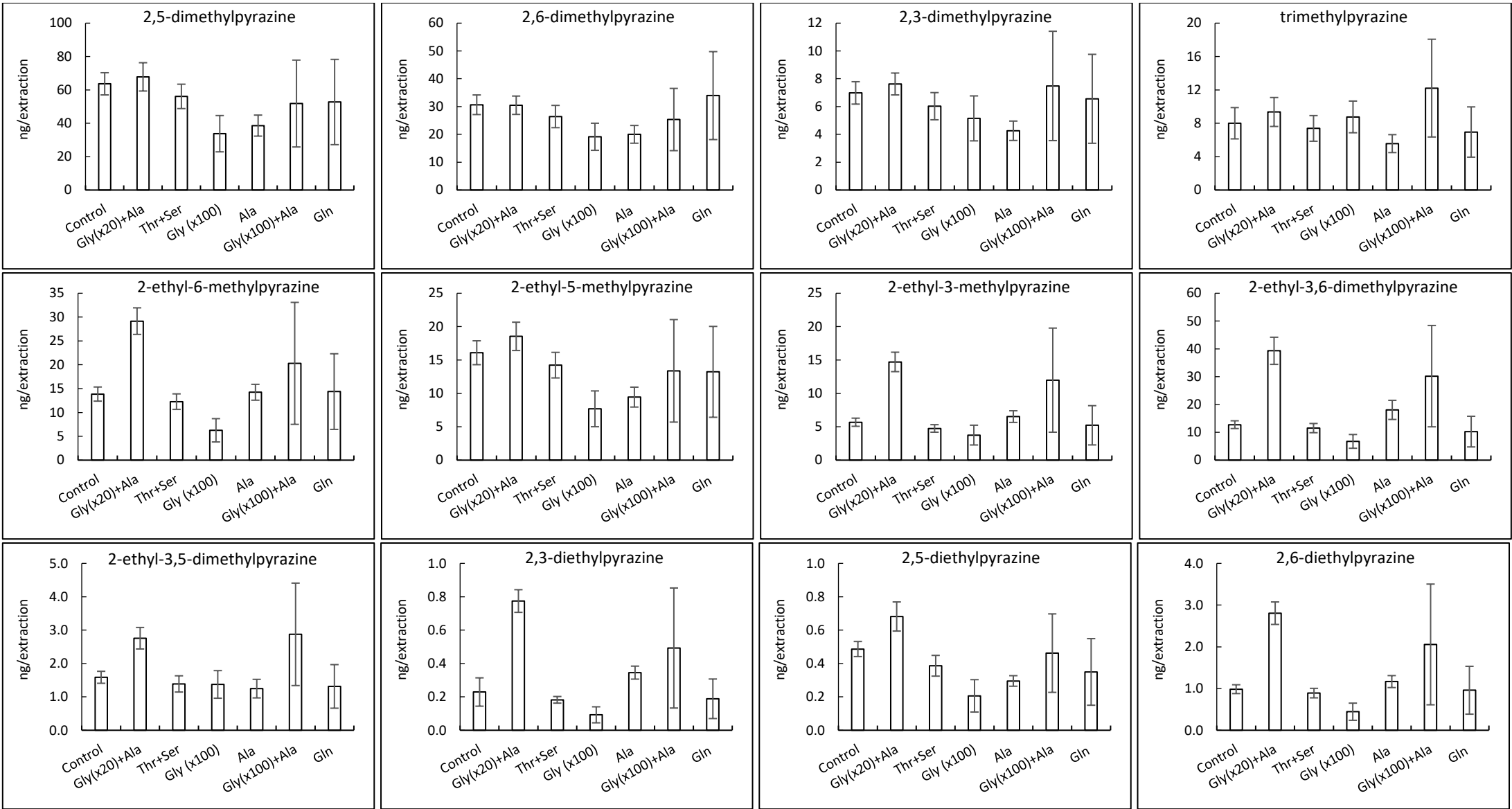
Figure S3. Formation of 2-ethyl-3,6-dimethylpyrazine and 2-ethyl-3,5-dimethylpyrazine

methylglyoxal

-2H$_2$O

methylglyoxal

-H$_2$O   CH$_3$CH=O from Alanine

2-ethyl-3,6-dimethylpyrazine

2,3-pentanedione

-2H$_2$O [O]

methylglyoxal

2-ethyl-3,5-dimethylpyrazine

Table S1. Concentrations of free amino acids (mg/kg) and reducing sugars (g/kg) in potato flake and the final concentration (mg/kg) of added amino acids in the recipes on potato flake basis

Amino acid	Concentration (mg/kg)	Gly(x20)+Ala	Gly(x100)+Ala	Met+Ile+Leu+Val	Gly(x100)+Ala+Met+Ile+Leu+Val	AA (x2)	2,3-PD	2,3-PD+Met+Ile+Leu+Val+Phe	Gln	Gly	Lys	Arg	Met	Val	Ala	Pro	Ser	Ile	Leu	Thr	Gly (x20)	Gly (x20)+Ala	Gly (x20)+Val	Gly (x20)+Thr	Thr+Ser	Gly (x100)	Gly (x100)+Ala
	Potao flake			Recipes in Figure 1			Recipes in Figure 2														Recipes in Figure S1 and S2						
Ala	531±1.3	2655	2655		2655	1593									2655							2655					2655
Arg	66±18.2					198						330															
Asn	2552±36.4																										
Asp	585±27.5					1755																					
Cys	nd*																										
GABA	580±4.3																										
Gln	401±2.8					2005	2005		2005																		
Glu	952±31.9					2856																					
Gly	18±0.6	378	1818		1818	54				90											378	378	378	378		1818	1818
His	90±0.4					270																					
Ile	157±3.3			785	785	471		785										785									
Leu	168±5.9			840	840	504		840											840								
Lys	35±5.3					105					175																
Met	107±0.2			535	535	321		535					535														
Phe	223±3.1					669		1115																			
Pro	97±1.1					291										485											
Ser	68±0.6					204											340								340		
Thr	98±0.2					294														490				490	490		
Trp	67±2.1					201																					
Tyr	250±2.0					750																					
Val	235±1.6			1175	1175	705		1175						1175									1175				

Sugar	Concentration (g/kg)
Glucose	6.7±0.24
Fructose	3.7±0.04

*nd: not detected

Table S2. Effect of amino acid addition on the formation of volatile Maillard reaction products (ng/extraction) during baking at 160 ˚C for 6 min followed by drying at 130 ˚C for 10 min (unless indicated amounts of added amino acids are 4-times the naturally occurring levels) (Within a row, values with the same lowercase letters are not statistically different at p=0.05.
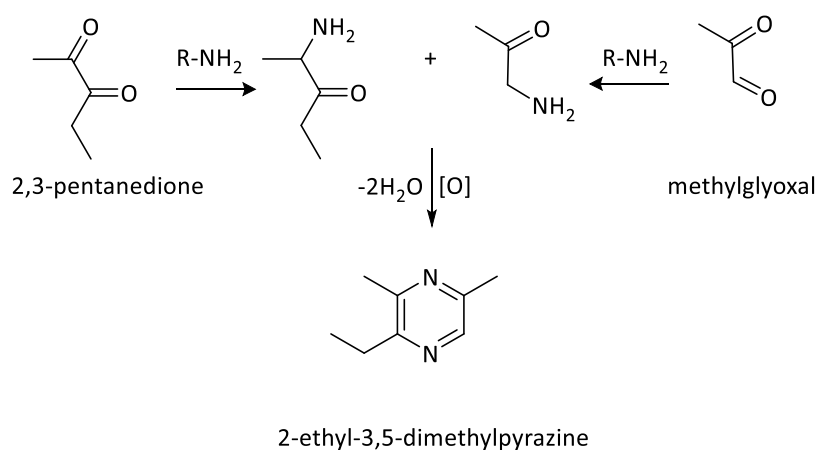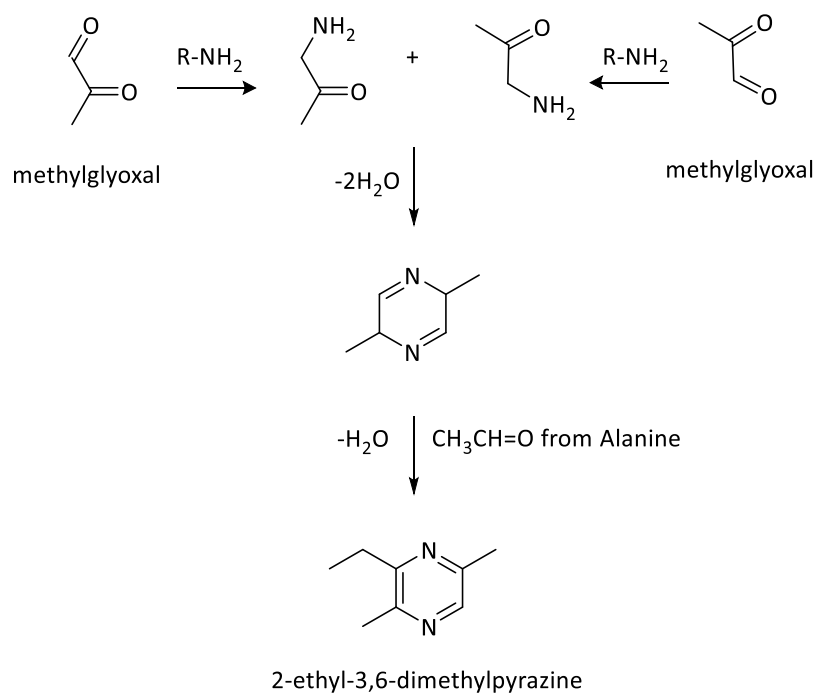
	r threshold in ;/L)ø	ol	20)+Ala	100)+Ala	le+Leu+Val	100)+Ala+ Met+Ile+Leu+Val	ino acids (×2) except Asn
2-Methylpropanal [A]		902	85±19 [c]	89±15 [c]	89±15 [c]	456±218 [a]	362±83 [ab]	250±39 [bc]
2-Methylbutanal [A]		1003	197±33 [d]	193±25 [d]	152±52 [d]	1066±189 [a]	875±129 [b]	506±61 [c]
3-Methylbutanal [A]		1008	166±24 [b]	165±11 [b]	133±39 [b]	859±146 [a]	692±105 [a]	429±33 [ab]
Methional [A]		1465	25±8 [d]	24±3 [d]	22±8 [d]	149±5 [a]	117±19 [b]	59±13 [c]
Phenylacetaldehyde [A]		1634	175±46 [b]	164±6 [b]	127±30 [b]	161±14 [b]	120±22 [b]	531±79 [a]
2,3-Butanedione [A]		1057	37±4.5 [abc]	39±2.2 [ab]	45±10 [a]	34±5.4 [bc]	37±5.9 [abc]	28±2.3 [c]
2,3-Pentanedione [A]		1130	26±4.4 [ab]	31±2.8 [a]	28±8.9 [a]	23±2.9 [ab]	24±4.4 [ab]	17±1.6 [b]
Dimethyl disulfide [A]		1136	44±6.3 [cd]	39±4.2 [cd]	32±7.0 [d]	88±19 [ab]	106±26 [a]	64±9.2 [bc]
Dimethyl trisulfide [A]		1387	5.2±2.4 [b]	4.5±1.1 [b]	6.2±2.5 [b]	7.3±0.3 [b]	13±2.9 [a]	7.4±1.6 [b]
Pyrazine [A]		1258	3.1±1.1 [a]	3.1±0.70 [a]	2.3±0.59 [a]	3.5±0.76 [a]	2.7±0.33 [a]	2.4±0.52 [a]
Methylpyrazine [A]	>2000	1296	24±8.0 [ab]	27±6.3 [a]	20±7.1 [ab]	29±6.1 [a]	23±3.5 [ab]	14±3.9 [b]
2,5-Dimethylpyrazine [A]	1820	1343	21±6.7 [ab]	30±8.5 [a]	25±11 [ab]	30±3.6 [a]	33±3.7 [a]	13±4.7 [b]
2,6-Dimethylpyrazine [A]	1720	1348	12±3.5 [ab]	15±3.3 [a]	13±5.2 [ab]	14±0.66 [ab]	15±2.4 [a]	7.9±2.1 [b]
Ethylpyrazine [A]	>2000	1351	13±4.6 [ab]	14±3.0 [ab]	9±3.1 [ab]	16±4.2 [a]	11±2.3 [ab]	8.9±2.3 [b]
2,3-Dimethylpyrazine [A]	880	1363	2.5±0.81 [bc]	3.3±0.79 [ab]	3.4±1.3 [ab]	3.2±0.40 [ab]	4.0±0.58 [a]	1.6±0.48 [c]
2-Ethyl-6-methylpyrazine [A]		1393	4.2±1.5 [bc]	10±2.8 [a]	7.6±3.0 [ab]	5.2±1.1 [bc]	9.6±1.7 [a]	3.6±1.2 [c]
2-Ethyl-5-methylpyrazine [A]		1398	5.6±1.8 [ab]	7.8±2.1 [a]	6.2±2.6 [ab]	7.9±1.5 [a]	8.6±1.1 [a]	4.1±1.4 [b]
2-Ethyl-3-methylpyrazine [A]	35	1411	1.7±0.61 [b]	5.0±1.5 [a]	4.3±1.7 [a]	2.1±0.48 [b]	5.6±1.0 [a]	1.3±0.44 [b]
Trimethylpyrazine [A]	50	1412	3.8±1.1 [cd]	5.4±1.4 [bc]	6.8±2.7 [ab]	5.1±0.30 [bcd]	8.8±0.90 [a]	2.5±0.80 [d]
2,6-Diethylpyrazine [A]	1.7	1437	0.30±0.11 [c]	0.93±0.27 [a]	0.71±0.29 [ab]	0.37±0.11 [bc]	0.96±0.18 [a]	0.30±0.10 [c]
2-Ethenylpyrazine [B]	>2000	1444	2.0±0.66 [ab]	2.0±0.34 [ab]	1.3±0.44 [b]	2.3±0.55 [a]	1.6±0.32 [ab]	1.2±0.32 [b]
2-Ethyl-3,6-dimethylpyrazine [A]	3.6	1448	4.5±1.4 [b]	16±4.9 [a]	14±6.2 [a]	6.5±1.1 [b]	20±2.5 [a]	4.4±1.7 [b]
2-Ethyl-3,5-dimethylpyrazine [A]	0.011	1464	0.59±0.18 [c]	1.2±0.32 [bc]	1.4±0.59 [ab]	0.88±0.17 [bc]	1.9±0.28 [a]	0.63±0.20 [c]
2,3-Diethylpyrazine [A]	6.6	1456	0.10±0.08 [bc]	0.25±0.07 [a]	0.18±0.07 [ab]	0.08±0.03 [bc]	0.27±0.07 [a]	0.06±0.02 [c]
2,5-Diethylpyrazine [A]	74	1462	0.17±0.05 [bc]	0.28±0.09 [ab]	0.18±0.08 [abc]	0.24±0.06 [abc]	0.30±0.07 [a]	0.13±0.04 [c]
2-Ethenyl-6-methylpyrazine [B]		1492	7.0±2.1 [ab]	9.2±1.6 [a]	7.6±2.8 [ab]	8.3±0.9 [ab]	8.3±1.2 [ab]	5.3±1.5 [b]
2-Ethenyl-5-methylpyrazine [B]		1499	1.6±0.47 [ab]	1.9±0.42 [a]	1.5±0.58 [ab]	2.1±0.41 [a]	1.9±0.26 [a]	1.0±0.27 [b]
2-Ethenyl-3,5(6)-dimethylpyrazine [B]	0.012(870)	1532	1.4±0.39 [bc]	2.0±0.44 [ab]	1.6±0.70 [abc]	1.9±0.34 [ab]	2.4±0.41 [a]	0.94±0.23 [c]
Isopropenylpyrazine [B]		1604	0.21±0.06 [a]	0.21±0.04 [a]	0.21±0.04 [a]	0.21±0.02 [a]	0.24±0.02 [a]	0.22±0.02 [a]
2-furfural [A]		1472	39±8.5 [a]	45±3.9 [a]	42±10.6 [a]	42±1.7 [a]	43±5.1 [a]	40±8.4 [a]

* LRI: Linear retention indices on Phenomenex ZB-Wax column (30 m × 250 μm i.d.× 1 μm), ø Values were taken from Wagner, R., Czerny, M., Bielohradsky, J., & Grosch, W. (1999). Structure odour-activity relationships of alkylpyrazines. Z. Lebensm. Unters. Forsch., 208(5-6), 308-316.

A : Identification was confirmed by LRI (authentic compounds) and mass spectra (NIST library)

B : Identification was confirmed by LRI (literature) and mass spectra (NIST library)

Table S3. Effect of whey protein hydrolysate (WPH), also together with 2,3-pentanedione and 3,4-hexanedione, on the formation of volatile Maillard reaction products (ng/extraction)

	LRI*	Control	WPH	WPH+2,3-PD	WPH+3,4-HD
2-Methylpropanal	902	77±11 [a]	87±6.2 [a]	108±17 [a]	81±36 [a]
2-Methylbutanal	1003	145±3.51 [a]	161±35.3 [a]	176±17 [a]	167±24 [a]
3-Methylbutanal	1008	144±3.84 [a]	137±28.6 [a]	145±12 [a]	138±18 [a]
Methional	1465	11±2.0 [a]	11±2.8 [a]	13±2.4 [a]	12±1.5 [a]
Phenylacetaldehyde	1634	88±2.0 [a]	77±14 [a]	90±12 [a]	88±10 [a]
2,3-Butanedione	1057	32±3.1 [a]	35±4.6 [a]	41±1.8 [a]	37±7.8 [a]
2,3-Pentanedione	1130	13±0.34 [b]	21±6.9 [b]	260±32 [a]	26±5 [b]
Dimethyl disulfide	1136	17±1.29 [b]	57±2.82 [a]	66±11 [a]	57±12 [a]
Dimethyl trisulfide	1387	1.9±0.39 [b]	4.2±0.47 [ab]	5.8±2.4 [a]	5±0.37 [a]
Pyrazine	1258	1.8±0.16 [a]	2.3±0.53 [a]	2.6±0.41 [a]	2±0.45 [a]
Methylpyrazine	1296	10±1.0 [b]	21±8.1 [ab]	27±8.2 [b]	22±6.0 [ab]
2,5-Dimethylpyrazine	1343	10±1.5 [b]	34±11 [a]	42±13 [a]	37±11 [a]
2,6-Dimethylpyrazine	1348	6.5±1.1 [b]	10±3.1 [ab]	12±2.9 [a]	11±2.6 [ab]
Ethylpyrazine	1351	4.3±0.72 [b]	7.7±3.3 [ab]	10±3.4 [a]	8±2.4 [ab]
2,3-Dimethylpyrazine	1363	1.1±0.13 [b]	1.8±0.60 [ab]	2.3±0.69 [a]	1.9±0.52 [ab]
2-Ethyl-6-methylpyrazine	1393	1.5±0.24 [b]	3.0±1.1 [ab]	4.0±1.4 [a]	3.2±0.94 [ab]
2-Ethyl-5-methylpyrazine	1398	2.2±0.34 [b]	7.5±2.7 [a]	9.5±3.2 [a]	8.3±2.4 [a]
2-Ethyl-3-methylpyrazine	1411	0.53±0.10 [b]	1.1±0.45 [b]	2.8±0.91 [a]	1.2±0.36 [b]
Trimethylpyrazine	1412	1.9±0.32 [b]	4.9±1.3 [a]	5.8±1.6 [a]	5.6±1.3 [a]
2,6-Diethylpyrazine	1437	0.10±0.02 [b]	0.19±0.07 [ab]	0.28±0.10 [a]	0.22±0.05 [ab]
2-Ethenylpyrazine	1444	0.70±0.09 [a]	1.1±0.41 [a]	1.4±0.42 [a]	1.1±0.32 [a]
2-Ethyl-3,6-dimethylpyrazine	1448	1.6±0.26 [b]	4.5±1.5 [ab]	6.7±2.3 [a]	5.0±1.4 [a]
2-Ethyl-3,5-dimethylpyrazine	1464	0.30±0.05 [c]	0.89±0.26 [bc]	2.1±0.64 [a]	1.0±0.26 [b]
2,3-Diethylpyrazine	1456	0.02±0.01 [b]	0.04±0.02 [b]	0.06±0.02 [b]	0.32±0.08 [a]
2,5-Diethylpyrazine	1462	0.06±0.00 [b]	0.19±0.07 [ab]	0.31±0.11 [a]	0.21±0.06 [a]
2,3-Diethyl-5-methylpyrazine	1485	0.04±0.01 [b]	0.10±0.04 [b]	0.15±0.06 [b]	0.60±0.12 [a]
2-Ethenyl-6-methylpyrazine	1492	3.4±0.54 [b]	6.2±1.7 [ab]	7.4±1.7 [a]	6.7±1.7 [a]
2-Ethenyl-5-methylpyrazine	1499	0.60±0.09 [b]	1.5±0.57 [a]	1.9±0.64 [a]	1.6±0.45 [a]
2-Ethenyl-3,5(6)-dimethylpyrazine	1532	0.60±0.06 [b]	1.8±0.56 [a]	2.2±0.69 [a]	1.9±0.46 [a]
2-furfural	1472	30±5.0 [a]	28±4.0 [a]	29±1.7 [a]	29±4.3 [a]

Within a row, values with the same lowercase letters are not statistically different at p=0.05.

* LRI: Linear retention indices on Phenomenex ZB-Wax column (30 m × 250 μm i.d.× 1 μm)

Table S4. Sensory characteristics of the samples defined by panellist and their reference materials

Attribute	Reference Material
Aroma	
Overall aroma intensity	
Baked	Crust under skin of jacketed potato
Boiled potato	Boiled potato
Fatty	Cooked oil
Sweet	
Cheesy	Mild cheddar
Taste	
Sweet	
Salty	
Bitter	
Umami	
Flavour	
Overall flavour intensity	
Baked	Crust under skin of jacketed potato
Boiled potato	Boiled potato
Fatty	Cooked oil
Cheesy	Mild cheddar
Aftertaste	
Baked	Crust under skin of jacketed potato
Boiled potato	Boiled potato
Sweet	
Salty	
Umami	
Fatty	Cooked oil
Cheesy	Mild cheddar