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Effects of variety and nutrient availability on the acrylamide-forming potential of rye grain

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ABSTRACT

Acrylamide is a probable human carcinogen that forms in plant-derived foods when free asparagine and reducing sugars react at high temperatures. The identification of rye varieties with low acrylamide-forming potential or agronomic conditions that produce raw material with low acrylamide precursor concentrations would reduce the acrylamide formed in baked rye foods without the need for additives or potentially costly changes to processes. This work compared five commercial rye varieties grown under a range of fertilisation regimes to investigate the effects of genotype and nutrient (nitrogen and sulphur) availability on the accumulation of acrylamide precursors. A strong correlation was established between the free asparagine concentration of grain and the acrylamide formed upon heating. The five rye varieties accumulated different concentrations of free asparagine in the grain, indicating that there is genetic control of this trait and that variety selection could be useful in reducing acrylamide levels in rye products. High levels of nitrogen fertilisation were found to increase the accumulation of free asparagine, showing that excessive nitrogen application should be avoided in order not to exacerbate the problem of acrylamide formation. This effect of nitrogen was mitigated in two of the varieties by the application of sulphur.

1. Introduction

The formation of acrylamide, a probable human carcinogen (Friedman, 2003), in cooked, plant-derived foods, came to light eleven years ago (Tareke et al., 2002). Since then, much has been learnt about the mechanisms involved in its formation, and methods have been developed to reduce its presence in foods. These have been reviewed by Claus et al. (2008) and compiled in a 'Toolbox' produced by Food Drink Europe: (http://www.fooddrinkeurope.eu/uploads/publications_documents/Toolboxfinal260911.pdf).

The Maillard reaction is the primary route by which acrylamide forms (Mottram et al., 2002; Stadler et al., 2002). At high temperatures, amino groups, principally those of free amino acids, react with reducing sugars to produce a plethora of compounds that impart colour, flavour and aroma. Only when the last stage of the reaction involves asparagine does it produce acrylamide (Mottram et al., 2002; Stadler et al., 2002; Zyzak et al., 2003), with 3-aminopropionamide a possible transient intermediate (Granvogl and Schieberle, 2006). The Maillard reaction is responsible for many of the characteristics associated with fried, baked and roasted foods that consumers demand and that define particular products and brands. It is important that when steps are taken to mitigate acrylamide formation, the aspects of the reaction responsible for the production of colours, flavours and aromas are retained to ensure that the quality of the final product is not affected.

Potentially, one of the most effective methods of acrylamide mitigation would be to reduce the accumulation of acrylamide precursors in plant material used for food production. The identification of genetic and environmental factors that affect precursor content is, therefore, an important approach (Halford et al., 2012a) and has been the objective of studies performed on several major crops, including wheat and potatoes (Amrein et al., 2003; Halford et al., 2012b; Martinek et al., 2009; Muttucumaru et al., 2008; Shepherd et al., 2010). These studies have also shown differences in the relationship between precursor concentration and acrylamide formation, with free asparagine concentration correlating closely with acrylamide-forming potential in wheat but with a more complex picture emerging for potato, in which different studies have shown reducing sugar concentration, free asparagine concentration,

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or the ratio of free asparagine to other free amino acids to be the most important parameter (reviewed by Halford et al., 2012a).

A previous study on rye (Curtis et al., 2010) analysed the acrylamide precursor content of a number of old and modern rye varieties grown in different locations across Europe within the EU FP6 HEALTHGRAIN diversity programme (Ward et al., 2008). The authors concluded that free asparagine concentration was the main determinant of the level of acrylamide that forms in heated rye flour, and proposed that commercial varieties should be screened for low free asparagine concentration in the grain. In contrast to wheat, the study also showed correlations between sugar concentrations and acrylamide formation.

Sulphur (S) deficiency is the most important factor affecting acrylamide-forming potential in wheat grain, with free asparagine concentration in the grain of severely S-deprived wheat being up to 30-times higher than in the grain of wheat supplied with adequate S (Curtis et al., 2009; Granvogl et al., 2007; Muttucumaru et al., 2006). A similar response occurs in barley (Shewry et al., 1983). Nitrogen (N) has the opposite effect, suggesting that some plants use free asparagine as a N store (Lea et al., 2007), particularly when there is insufficient S available for the efficient synthesis of storage proteins (Shewry et al., 2001). S availability also causes changes in the distribution of free asparagine in wheat grain, with more accumulating in the endosperm (white flour) fraction of S-deficient grain and therefore affecting more products (Shewry et al., 2009).

In the present study, five current commercial varieties of rye were compared when grown in the same location at the same time, with nine different combinations of S and N application. Free amino acid and sugar concentrations in the grain were determined and related to the amount of acrylamide that formed in heated flour, with the aim of assessing differences in acrylamide-forming potential between the varieties, the relationship between precursor concentration and acrylamide formation and the importance of nutrient availability.

2. Materials and methods

2.1. Trial design

The trial used five rye varieties (Agronom, Askari, Festus, Fugato and Rotari) with seed provided by Saaten Union (Suffolk, UK). It was sown at the Rothamsted Farm site at Woburn, Bedfordshire, UK, which has a sandy loam soil with very poor nutrient retention (for example, soil sulphur (S) concentrations range from 0.5 to 1.8 mg kg^{-1}) (Riley et al., 2002). S was applied as gypsum (calcium sulphate dihydrate) to give 0, 15 or 40 kg S ha^{-1}, in combination with nitrogen (N) applied as ammonium nitrate to give 1, 100 or 200 kg N ha^{-1}, resulting in nine different combinations of N and S. The trial was designed in a criss-cross layout, with the five varieties grown as main plots and nutrient treatments applied in rows and columns within each main plot. The main plots each comprised nine individual plots of 1.8 × 4 m and were separated by 3 m surrounds sown with sorghum to reduce cross-pollination between the varieties. Two blocks were used to provide biological replication. The trial was harvested in August 2010 and 5 g of grain from each plot was milled to fine, wholemeal flour for analysis.

2.2. Concentrations of free amino acids

Flour (0.5 g ± 0.005 g) was added to 10 mL of 0.01 N HCl and stirred for 15 min. The suspension was left to settle for 15 min at room temperature and an aliquot (1.5 mL) was centrifuged at 7200 *g* to produce a clear extract. Amino acids were derivatised using the EZ: Faast free amino acid kit (Phenomenex, Torrance, CA). Gas chromatography–mass spectrometry (GC–MS) analysis of the derivatised samples was carried out using an Agilent 6890 GC-5975-MS system (Agilent, Santa Clara, CA) in electron impact mode, as described by Elmore et al. (2005). An aliquot of the derivatised amino acid solution (1 μL) was injected at 250 °C in split mode (20:1) onto a Zebron ZB-AAA capillary column (10 m × 0.25 mm; 0.25 μm film thickness). The oven temperature was held at 110 °C for 1 min and then increased at 30 °C min^{-1} to 310 °C. The transfer line and ion source were maintained at 320 and 230 °C respectively; carrier gas flow rate was kept constant throughout the run at 1.1 mL min^{-1}. Amino acid standards were provided with the EZ: Faast kit and were >99% pure (Phenomenex). Separate calibration curves were calculated for each amino acid. The standards were also used before, during and after the analysis of each batch of samples to check that the machine was running correctly. Analyses of the data were performed using the Agilent 5975 system data analysis software.

2.3. Analysis of sugars

The concentrations of reducing sugars and sucrose in the flour samples were measured using a method adapted from Curtis et al. (2010). Flour (0.5 g ± 0.005 g) was added to 10 mL methanol/water (50% v/v) containing 100 mg L^{-1} trehalose as an internal standard. The flour suspension was stirred for 15 min and left to settle for a further 15 min. An aliquot of the solution was then centrifuged at 7200 *g* to produce a clear extract; this was then diluted four-fold in water and filtered through a 0.2 μm syringe filter into a vial. A Dionex ion chromatography system with a CarboPac PA1 column and pulsed amperometric detection (Thermo, Waltham, MA) was used to analyse the sugar content of each sample. The injection volume was 25 μL and the eluent was 65% water and 35% 4 M NaOH at a flow rate of 1 mL min^{-1}; at 11 min the eluent was changed to 50% water and 50% 4 M NaOH for the remainder of the run, the run ending at 18 min. The waveform of the pulsed amperometric detector was 400 ms at 0.1 V, 20 ms at −2.0 V, 10 ms at 0.6 V, and 60 ms at −0.15 V. Standard solutions of glucose, fructose, sucrose and maltose were used to produce calibration curves for quantification.

2.4. Analysis of acrylamide by liquid chromatography–mass spectrometry/mass spectrometry (LC–MS/MS)

Rye flour (0.5 g ± 0.005 g) was weighed into a 1 mL ampoule and heated at 160 °C for 20 min. The cooked flour was then added to 40 mL of water containing 2 μg of $^{13}C_3$-acrylamide as an internal standard and shaken for 20 min. Centrifugation of the sample at 15 °C produced a clear extract, 2 mL of which were filtered through a 0.2 μm syringe filter into a vial for analysis. LC–MS/MS was performed using an Agilent 1200 high-performance liquid chromatography (HPLC) system connected to an Agilent 6410 triple quadrupole mass spectrometer. Separation was performed using a 100 × 3.0 mm Hypercarb column protected by a KrudKatcher (Phenomenex, Torrance, CA) and a pre-column (Hypercarb 10 mm × 3.0 mm 5 μm particle size; Thermo, Waltham, MA) with 0.1% formic acid as the mobile phase with a flow rate of 0.3 mL min^{-1}. Most of the extract was not retained by the column and was eluted as waste before the acrylamide eluted at around 6 min. The transitions m/z 72 → 55 and m/z 72 → 27 were measured for acrylamide, and m/z 75 → 58 for $^{13}C_3$-acrylamide.

2.5. Measurements of total grain N and S

Measurements of total grain N and S were made by the Analytical Unit of the Soil Science Department, Rothamsted Research. Total grain N was determined according to the Dumas digestion method, using a LECO CNS 2000 combustion analyser (Leco, Stockport, UK). Total S content was determined using an Accuris inductively coupled plasma optical emission spectrometer