Food Chemistry 239 (2018) 304–313

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effects of variety, year of cultivation and sulphur supply on the accumulation of free asparagine in the grain of commercial wheat varieties

Tanya Y. Curtis [a], Stephen J. Powers [b], Ruiyun Wang [a,c], Nigel G. Halford [a,*]

[a] Plant Science Department, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, United Kingdom
[b] Computational and Analytical Sciences Department, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, United Kingdom
[c] Agronomy College, Shanxi Agricultural University, and Key Laboratory of Crop Gene Resources and Germplasm Enhancement on Loess Plateau, Ministry of Agriculture, Shanxi Key Laboratory of Genetic Resources and Genetic Improvement of Minor Crops, Institute of Crop Germplasms Resources of Shanxi Academy of Agricultural Sciences, Taiyuan City, Shanxi Province 030031, People's Republic of China

ARTICLE INFO

Article history:
Received 15 March 2017
Received in revised form 24 May 2017
Accepted 20 June 2017
Available online 21 June 2017

Chemical compounds studied in this article:
Acrylamide (PubChem CID: 6579)
L-Asparagine (PubChem CID: 6267)

Keywords:
Acrylamide
Asparagine
Food safety
Free amino acids
Processing contaminants
Wheat

ABSTRACT

Free asparagine concentration, which is the determining factor for acrylamide-forming potential in cereals, was measured in grain from wheat grown in field trials in the United Kingdom in 2011–2012 and 2012–2013. There were 25 varieties in 2012 and 59 in 2013, with eleven present in both trials. The trials were split-plot, with half of each plot supplied with sulphur and the other half not. The varietal means (mmol per kg) for free asparagine in the sulphur-fed wheat ranged from 1.521 to 2.687 in 2011–2012 and 0.708 to 11.29 in 2012–2013. Eight varieties were identified as having consistently low free asparagine concentration. There was a differential response of varieties to sulphur, and much higher levels of free asparagine in 2012–2013 versus 2011–2012. Given the short commercial lifespan of some wheat varieties, it is concluded that information on free asparagine concentration should be made available when a variety is launched.

1. Introduction

Food processing and cooking bring about substantial changes in food composition, including the production of substances that are not present in the raw food. These changes may be necessary to make the food edible and/or palatable, and many of the substances that are produced are responsible for the colours, flavours, and aromas that define food types and distinguish brands. However, some of the substances that are produced fall into the category of processing contaminant, defined as a substance that is produced in a food when it is cooked or processed, is not present or is present at much lower concentrations in the raw, unprocessed food, and is undesirable either because it has an adverse effect on product quality or because it is potentially harmful (Curtis, Postles, & Halford, 2014).

A processing contaminant that is proving to be an increasingly difficult problem for the food industry is acrylamide, which forms within the Maillard reaction during the frying, baking, roasting or high-temperature processing of cereals, potatoes, coffee and other plant-derived raw materials, with all major cereal products, including bread, crispbread, breakfast cereals, cakes and biscuits, being affected (European Food Safety Authority, 2011). Acrylamide is classed as a probable (Group 2a) human carcinogen by the International Agency for Research on Cancer (1994), based on its action in rodents, and also has reproductive and neurotoxicological effects at high doses (Friedman, 2003).

The European Food Safety Authority (EFSA) Expert Panel on Contaminants in the Food Chain (CONTAM) has stated that it considers the margin of exposure for acrylamide (the ratio of the level at which a small but measurable effect is observed to the estimated exposure dose) to be low enough to cause concern that dietary

* Corresponding author.
E-mail addresses: tanyayankova@msn.com (T.Y. Curtis), stephen.powers@rothamsted.ac.uk (S.J. Powers), wry925@126.com (R. Wang), nigel.halford@rothamsted.ac.uk (N.G. Halford).

http://dx.doi.org/10.1016/j.foodchem.2017.06.113
0308-8146/

acrylamide could have neoplastic (tumor-inducing) effects (European Food Safety Authority Panel on Contaminants in the Food Chain (CONTAM), 2015). As a result, the European Commission is reviewing its risk management measures for dietary acrylamide intake, which since 2011 have been based on non-mandatory 'Indicative Values' for the presence of acrylamide in foods (European Commission, 2013). In the USA, the Food and Drug Administration (FDA) has so far stopped short of issuing advice or restrictions on levels of acrylamide in food, but has issued an 'action plan' for the food industry (Food and Drug Administration, 2016).

Acrylamide forms predominantly *via* a Strecker-type degradation of free (i.e. soluble, non-protein) asparagine by highly reactive carbonyl compounds produced within the Maillard reaction (Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002; Zyzak et al., 2003), although other routes for its formation have been proposed (Claus, Weisz, Schieber, & Carle, 2006; Granvogl and Schieberle, 2006). The production of carbonyl compound intermediates within the Maillard reaction also involves reducing sugars, such as glucose, fructose and maltose, and other free amino acids, which means that the concentrations of these metabolites as well as free asparagine may affect acrylamide formation, depending on their relative concentrations (Muttucumaru et al., 2017). However, in wheat (*Triticum aestivum*) and rye (*Secale cereale*), and probably other cereals, free asparagine concentration is the major determinant of acrylamide-forming potential (Curtis & Halford, 2016; Curtis, Powers, & Halford, 2016; Curtis et al., 2009; Curtis et al., 2010; Granvogl, Wieser, Koehler, Von Tucher, & Schieberle, 2007; Muttucumaru et al., 2006; Postles, Powers, Elmore, Mottram, & Halford, 2013).

The challenge for the food industry is to reduce acrylamide levels while retaining the colours, flavours and aromas that define products and brands and are demanded by consumers. The methods that the industry has developed to reduce acrylamide formation have been shared in the 'Acrylamide Toolbox', published by FoodDrinkEurope (2013). For cereal-based products, the Toolbox recommends the use of grain with low free asparagine content, and switching from a high free asparagine to a low free asparagine variety would be easier and cheaper than doing anything else, while any change to processing would be more effective from a low asparagine starting point. However, this is not as simple as it sounds, because free asparagine concentration in many plant tissues, including cereal grain, is affected by environmental factors (E) that are unpredictable and beyond the control of producers (Halford, Curtis, Chen, & Huang, 2015; Lea, Sodek, Parry, Shewry, & Halford, 2007). Nevertheless, crop management measures have been shown to be effective, and in the case of wheat this means ensuring that the crop is supplied with sufficient sulphur during cultivation and is protected from disease (Curtis & Halford, 2016; Curtis et al., 2009; Curtis, Halford, Powers, McGrath, & Zazzeroni, 2014; Curtis et al., 2016; Granvogl et al., 2007; Martinek et al., 2009; Muttucumaru et al., 2006;). Genetic factors (G) also play a part, on their own and interacting with the environment (G × E) (Corol et al., 2016; Curtis et al., 2009, 2010; Curtis, Powers, & Halford, 2016; Curtis & Halford, 2016; Granvogl et al., 2007; Muttucumaru et al., 2006; Postles, Powers, Elmore, Mottram, & Halford, 2013), and key questions for wheat breeders as they begin to address the acrylamide issue include: 1) how wide is the range in free asparagine concentration in different wheat genotypes? and 2) Is it possible to identify varieties that are consistently low in free asparagine concentration in the grain in a range of environments? In the present study we address these questions by comparing elite wheat varieties from the UK, grown in field trials in 2011–2012 and 2012–2013, with and without sufficient sulphur.

2. Materials and methods

2.1. Field trials

Field trails of winter wheat were carried out at the Rothamsted Farm site at Woburn, Bedfordshire, United Kingdom (UK), in 2011–2012 and 2012–2013. There were 73 varieties and genotypes altogether over the two trials, 25 in 2011–2012 and 59 in 2012–2013, with 11 varieties being present in both trials. The trials comprised a split-plot in three randomized blocks (replicates) design with sulphur applied to one half of each plot and not the other. In the 2012–2013 trial, genotype SR3, which had previously been shown to have a relatively low free asparagine concentration in the grain, was replicated twice in each block. The 2011–2012 trial was fertilized with Nitram® (ammonium nitrate: CF Fertilisers, Chester, UK) on the 28th of March 2012 at a rate of 116 kg/ha (40 kg/ha nitrogen), and on the 10th of May 2012 at a rate of 262 kg/ha (90 kg/ha nitrogen). Sulphur was applied by hand on the 23rd of April 2012 as agricultural gypsum (calcium sulphate dihydrate) (Saint-Gobain, British Gypsum, Loughborough, UK) at a rate of 40 kg sulphur per hectare (100 kg per hectare SO_3 equivalent). Planting of the 2012–2113 trial was delayed due to heavy rain, and Nitram® was not applied until 25th April 2013, at a rate of 174 kg/ha (60 kg/ha nitrogen), and then on the 9th of May 2013 at a rate of 232 kg/ha (80 kg/ha nitrogen). Sulphur was again applied by hand on the 26th of April 2013 as agricultural gypsum at a rate of 40 kg sulphur per hectare. Grain was harvested in August 2012 and 2013, and samples milled to fine, wholemeal flour for analysis.

2.2. Free amino acid concentrations

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* for 15 min to produce a clear extract. Amino acids were derivatised using the EZ: Faast free amino acid kit (Phenomenex, Torrance, CA) using the protocol provided in the manufacturer's manual with the following modifications: a second wash step was included with Reagent 2, and the sample was vortexed for 10 s after addition of Reagent 4, then rested for 2 min before vortexing again for 16 s before derivatisation. 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 previously (Elmore, Koutsidis, Dodson, Mottram, & Wedzicha, 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 °C 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 200 samples to check that the machine was running correctly. Two technical replicates per biological replicate sample were assayed for the 2011–2012 trial, and three per biological replicate sample for the 2012–2013 trial. Analyses of the data to extract quantities (mmol/kg) of each amino acid using the calibration curves were performed using the Agilent 5975 system data analysis software.