Accounting for environmental variation in the free asparagine content of wheat grain

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To link to this article DOI: http://dx.doi.org/10.1016/j.jfca.2023.105333

Publisher: Elsevier

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Commentary

Accounting for environmental variation in the free asparagine content of wheat grain

Abstract

The free (soluble, non-protein) asparagine content of wheat grain can vary massively due to environmental factors and agronomic differences in how the crop is grown. This is concerning because free asparagine concentration is the key determinant of acrylamide formation in wheat-based food products, yet it is not clear how different environmental conditions and some agronomic practices impact grain asparagine content. A key aspect of keeping free asparagine levels low in wheat grain in the future lies in improving our understanding of the many environmental and crop management components that impact free asparagine accumulation during cultivation. We also consider the emerging technology of monitoring free asparagine accumulation through crop imaging.

Main text

The formation, via the Maillard reaction, of the probable human carcinogen acrylamide in certain cooked foods was first reported in 2002 (Tareke et al., 2002), and strategies to reduce the potential for its formation continue to be of importance (Peivasteh-Roudsari et al., 2023). As the key determinant of acrylamide levels in wheat-based food products, the free asparagine content of wheat grain has been the subject of much investigation. The evolving regulations concerning acrylamide levels in food, with the EU reportedly looking at imposing Maximum Levels, above which it would be illegal to sell a food product, possibly in 2023, mean that the free asparagine content of wheat grain will likely have even greater importance to the food industry in the years to come (European Commission, 2023). However, environmentally-induced variation in grain free asparagine content (represented in the variation due to trial year and site) remains mostly unaccounted for. Many possible causes for this environmental variation have been proposed, such as differences in weather, disease pressure, and soil type of the trial site. There will always be a risk of grain being produced with unexpectedly high concentrations of free asparagine while this variation remains poorly understood.

30   Figure 1 shows data from a number of field trials that measured free asparagine

31   concentrations in wheat grain. Sulphur deficiency has been shown to cause a massive increase

32   in free asparagine concentration in the grain (reviewed by Raffan and Halford, 2019). However,

33   there was no significant effect of sulphur deficiency on grain free asparagine content in a field

34   trial conducted in 2011–2012 at Woburn in the UK (shown in Figure 1 as 11/12 Woburn trial),

35   whereas a pronounced effect was observed at the same site in 2012–2013 (12/13 Woburn in

36   Figure 1), meaning that there was a significant year by treatment interaction ($p < 0.001$, F-test)

37   (Curtis et al., 2018). The reason for this was suggested to have been an effect of the weather,

38   the spring and summer of 2012 being unusually wet in the UK, but the authors conceded that

39   this explanation was entirely speculative. Morris et al. (2022) also did not observe any impact

40   of variable sulphur fertilisation on grain asparagine content in a series of trials across different

41   sites and years, although there were other differences between the trials. In this case, the lack

42   of a sulphur effect was attributed to the intrinsic sulphur sufficiency of the soil at the trial sites.

43   On the other hand, Wilson et al. (2020) observed extremely high concentrations of free

44   asparagine content in response to sulphur deficiency in trials undertaken in Kansas.

45   This influence of trial location and year has been quantified in some cases: Malunga et

46   al. (2021), for example, found that in their study of grain free asparagine content across two

47   trial sites, 80% of the observed variation in free asparagine was due to location. Xie et al. (2021)

48   similarly found that 44.3% of the observed variation in grain free asparagine content across

49   four different field trials was attributable to environmental effects (trial year and/or location).

50   The same study also noted that sulphur fertilisation did not have any impact at trial sites where

51   sulphur was already plentiful in the soil. It is notable from this comparison of trials that those

52   studies which show the greatest variability in free asparagine levels are those that test different

53   application rates of sulphur.

54   Many of the studies shown in Figure 1 detected significant effects of genotype within

55   their trials, often with significant G × E interactions (Curtis et al., 2016; Curtis et al., 2018;

56   Wilson et al., 2020; Oddy et al., 2022), emphasising the need to consider genotype alongside

57   environment and the interaction between the two. The importance of genotypic effects on free

58   asparagine was recently demonstrated through the trialling of wheat that had been edited to

59   have non-functional asparagine synthetase 2 genes (Raffan et al., 2023), which showed large

60   decreases relative to genotypes with functional alleles. The use of such gene-edited genotypes

61   is likely to increase in the future in the UK due to the recent passing of the UK Genetic

62   Technology act (UK Parliament, 2023).

63

64   **Fig. 1 here.**

65

66   These data make it clear that the development of low free asparagine varieties must be
67   accompanied by further research on the effects of the environment and crop management, and
68   the G × E interaction, or the G × E × M interaction if environmental and management effects
69   are separated. This is made more important by the fact that environment/management factors
70   may have differential effects on different varieties, meaning that varietal rankings break down
71   under some conditions. This is true for both sulphur deficiency and disease control (Curtis et
72   al., 2016; 2018). We encourage wheat breeders to develop low asparagine wheat varieties, but
73   this will have to be accompanied by further research into the other causes of environmental
74   variation, involving field trials measuring all relevant factors that could impact grain free
75   asparagine content. This would require measurements of weather, disease pressure, soil
76   nutrients and fertiliser application over several sites and years. By breaking down the effects
77   of the environment into constituent parts, the amount of variation attributable to each
78   component could be measured. This could be done alongside glasshouse studies that introduced
79   individual, specific stressors to isolate and compare the effect each stressor had on grain free
80   asparagine content in a controlled environment. These experiments could be more easily
81   performed due to their smaller scale but, of course, conclusions drawn would have to be tested
82   in the field.

83   A more detailed understanding of the environmental factors that control grain free
84   asparagine content could enable food manufacturers to predict the grain free asparagine content
85   of their raw material, but it would take several years of trials for predictive models to be
86   developed. In the meantime, it may be possible to develop accessible imaging tools that could
87   predict grain free asparagine content from measurements of plants and seeds. We have used
88   multispectral measurements of plants in the field to predict the free asparagine content of grain,
89   with an accuracy of 71 %, whilst a combination of multispectral, fluorescence, and
90   morphological measurements of seeds could distinguish high and low free asparagine grain
91   with an even higher accuracy of 86 % (Oddy et al., 2023). However, a number of challenges
92   would need to be addressed if this approach were to become widely applicable. Firstly,
93   predictive models need to be built from many measurements of plants and seeds. By training
94   models on diverse datasets, predictions of free asparagine content would become accurate

95 across a range of different environments. This could be achieved by collecting imaging data

96 from a series of field trials in different locations and years, similar to those described above for

97 the study of environmental variation. Indeed, the simultaneous collection of imaging and

98 environmental variation data would greatly enhance our ability to predict grain free asparagine

99 content.

100 Of course, the ability to build such models relies on the assumption that stressors that

101 cause free asparagine to accumulate in the grain also leave a "signature" that can be detected

102 by imaging technology (an obvious example being the yellowing of the wheat canopy caused

103 by sulphur deficiency). Many stressors do cause such signatures (Lowe et al., 2017), but the

104 relationship between signatures of stress and grain free asparagine content requires further

105 research. The development of such models would have to assess the conditions under which

106 predictions were most accurate, and those in which the models would under- or over-estimate.

107 Another challenge facing the deployment of such a monitoring strategy is the

108 accessibility of imaging tools. Some labour-intensive forms of monitoring require the imaging

109 device to be held above the crop canopy to take a measurement. Similar multispectral

110 measurements can be taken with unmanned aerial vehicles (UAVs) (Feng et al., 2021), and this

111 would save time and labour. UAVs may not be accessible to every farmer, but this may be

112 something contract agronomists could help with. Alternatively, it may be possible to use

113 satellite data to estimate grain free asparagine content. The spatial resolution of satellite data

114 collection continues to improve (some satellites now have a resolution of 0.25 m^2) and some

115 satellites are able to collect multispectral data (Li et al., 2021). Access to and interpretation of

116 these data would still require specialist knowledge (as with other monitoring methods), but the

117 use of satellite data may require less input from the farmer in terms of time, labour, and money.

118 In conclusion, as regulations concerning the levels of acrylamide in food continue to

119 tighten, the need to understand why free asparagine accumulates in wheat grain will only

120 become more important. Further developing our ability to predict free asparagine content from

121 environmental variables and crop monitoring technology will enable the targeting of measures

122 to control free asparagine accumulation during cultivation and the proactive identification of

123 grain with high acrylamide-forming potential before it enters the food chain. It will also ensure

124 that maximum benefit is derived from low asparagine varieties as they become available.

125

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FIGURE
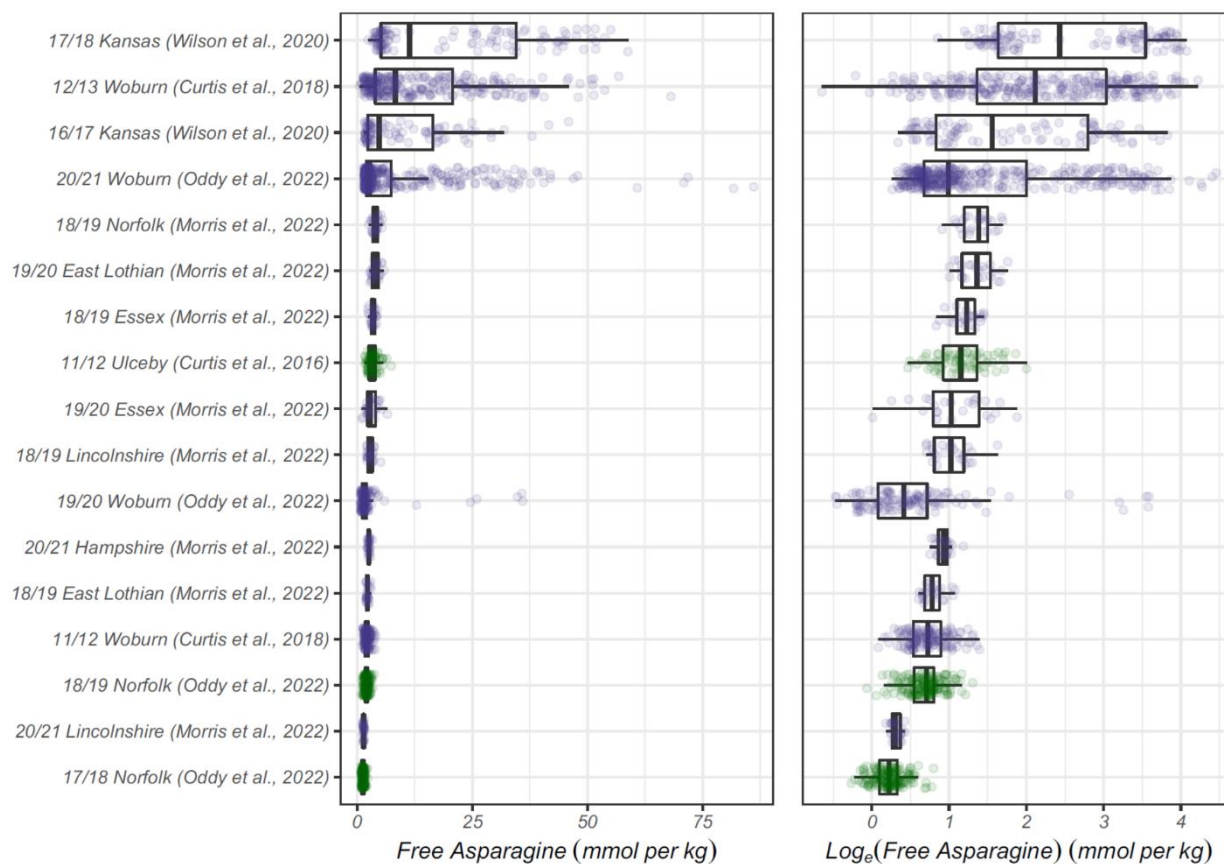
180   **Figure 1.** Free asparagine concentrations in wheat grain from 18 different field trials on raw

181   scale (left panel) and log$_e$ transformed (right panel). Trials testing different levels of sulphur

182   are shown in purple and those with consistent levels of fertiliser are shown in green. The trial

183   undertaken by Curtis et al. (2016) contained plots that were either treated or untreated with

184   fungicides. Boxes show the interquartile range and median values, with the whiskers showing

185   the largest or smallest value within 1.5 times below or above the 25th or 75th percentile,

186   respectively.

187