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

Article

Accepted Version

Oddy, J., Elmore, J. S. ORCID: https://orcid.org/0000-0002-2685-1773 and Halford, N. G. (2023) Accounting for environmental variation in the free asparagine content of wheat grain. Journal of Food Composition and Analysis, 120. 105333. ISSN 1096-0481 doi: https://doi.org/10.1016/j.jfca.2023.105333 Available at https://centaur.reading.ac.uk/111986/

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

Publisher: Elsevier

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.

Figure 1 shows data from a number of field trials that measured free asparagine concentrations in wheat grain. Sulphur deficiency has been shown to cause a massive increase in free asparagine concentration in the grain (reviewed by Raffan and Halford, 2019). However, there was no significant effect of sulphur deficiency on grain free asparagine content in a field trial conducted in 2011–2012 at Woburn in the UK (shown in Figure 1 as 11/12 Woburn trial), whereas a pronounced effect was observed at the same site in 2012–2013 (12/13 Woburn in Figure 1), meaning that there was a significant year by treatment interaction ($p < 0.001$, F-test) (Curtis et al., 2018). The reason for this was suggested to have been an effect of the weather, the spring and summer of 2012 being unusually wet in the UK, but the authors conceded that this explanation was entirely speculative. Morris et al. (2022) also did not observe any impact of variable sulphur fertilisation on grain asparagine content in a series of trials across different sites and years, although there were other differences between the trials. In this case, the lack of a sulphur effect was attributed to the intrinsic sulphur sufficiency of the soil at the trial sites. On the other hand, Wilson et al. (2020) observed extremely high concentrations of free asparagine content in response to sulphur deficiency in trials undertaken in Kansas.

This influence of trial location and year has been quantified in some cases: Malunga et al. (2021), for example, found that in their study of grain free asparagine content across two trial sites, 80% of the observed variation in free asparagine was due to location. Xie et al. (2021) similarly found that 44.3% of the observed variation in grain free asparagine content across four different field trials was attributable to environmental effects (trial year and/or location). The same study also noted that sulphur fertilisation did not have any impact at trial sites where sulphur was already plentiful in the soil. It is notable from this comparison of trials that those studies which show the greatest variability in free asparagine levels are those that test different application rates of sulphur.

Many of the studies shown in Figure 1 detected significant effects of genotype within their trials, often with significant G × E interactions (Curtis et al., 2016; Curtis et al., 2018; Wilson et al., 2020; Oddy et al., 2022), emphasising the need to consider genotype alongside environment and the interaction between the two. The importance of genotypic effects on free asparagine was recently demonstrated through the trialling of wheat that had been edited to have non-functional asparagine synthetase 2 genes (Raffan et al., 2023), which showed large decreases relative to genotypes with functional alleles. The use of such gene-edited genotypes is likely to increase in the future in the UK due to the recent passing of the UK Genetic Technology act (UK Parliament, 2023).

Fig. 1 here.

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

A more detailed understanding of the environmental factors that control grain free asparagine content could enable food manufacturers to predict the grain free asparagine content of their raw material, but it would take several years of trials for predictive models to be developed. In the meantime, it may be possible to develop accessible imaging tools that could predict grain free asparagine content from measurements of plants and seeds. We have used multispectral measurements of plants in the field to predict the free asparagine content of grain, with an accuracy of 71 %, whilst a combination of multispectral, fluorescence, and morphological measurements of seeds could distinguish high and low free asparagine grain with an even higher accuracy of 86 % (Oddy et al., 2023). However, a number of challenges would need to be addressed if this approach were to become widely applicable. Firstly, predictive models need to be built from many measurements of plants and seeds. By training models on diverse datasets, predictions of free asparagine content would become accurate across a range of different environments. This could be achieved by collecting imaging data from a series of field trials in different locations and years, similar to those described above for the study of environmental variation. Indeed, the simultaneous collection of imaging and environmental variation data would greatly enhance our ability to predict grain free asparagine content.

Of course, the ability to build such models relies on the assumption that stressors that cause free asparagine to accumulate in the grain also leave a “signature” that can be detected by imaging technology (an obvious example being the yellowing of the wheat canopy caused by sulphur deficiency). Many stressors do cause such signatures (Lowe et al., 2017), but the relationship between signatures of stress and grain free asparagine content requires further research. The development of such models would have to assess the conditions under which predictions were most accurate, and those in which the models would under- or over-estimate.

Another challenge facing the deployment of such a monitoring strategy is the accessibility of imaging tools. Some labour-intensive forms of monitoring require the imaging device to be held above the crop canopy to take a measurement. Similar multispectral measurements can be taken with unmanned aerial vehicles (UAVs) (Feng et al., 2021), and this would save time and labour. UAVs may not be accessible to every farmer, but this may be something contract agronomists could help with. Alternatively, it may be possible to use satellite data to estimate grain free asparagine content. The spatial resolution of satellite data collection continues to improve (some satellites now have a resolution of 0.25 m^2) and some satellites are able to collect multispectral data (Li et al., 2021). Access to and interpretation of these data would still require specialist knowledge (as with other monitoring methods), but the use of satellite data may require less input from the farmer in terms of time, labour, and money.

In conclusion, as regulations concerning the levels of acrylamide in food continue to tighten, the need to understand why free asparagine accumulates in wheat grain will only become more important. Further developing our ability to predict free asparagine content from environmental variables and crop monitoring technology will enable the targeting of measures to control free asparagine accumulation during cultivation and the proactive identification of grain with high acrylamide-forming potential before it enters the food chain. It will also ensure that maximum benefit is derived from low asparagine varieties as they become available.

References

Curtis, T. Y., Powers, S. J., & Halford, N. G. (2016). Effects of fungicide treatment on free amino acid concentration and acrylamide-forming potential in wheat. *Journal of Agricultural and Food Chemistry*, *64*(51), 9689-9696.

Curtis, T. Y., Powers, S. J., Wang, R., & Halford, N. G. (2018). Effects of variety, year of cultivation and sulphur supply on the accumulation of free asparagine in the grain of commercial wheat varieties. *Food Chemistry, 239*, 304-313.

European Commission. (2023). Acrylamide. https://food.ec.europa.eu/safety/chemical-safety/contaminants/catalogue/acrylamide_en

Feng, L., Chen, S., Zhang, C., Zhang, Y., & He, Y. (2021). A comprehensive review on recent applications of unmanned aerial vehicle remote sensing with various sensors for high-throughput plant phenotyping. *Computers and Electronics in Agriculture, 182*, 106033.

Li, D., Quan, C., Song, Z., Li, X., Yu, G., Li, C., & Muhammad, A. (2021). High-throughput plant phenotyping platform (HT3P) as a novel tool for estimating agronomic traits from the lab to the field. *Frontiers in Bioengineering and Biotechnology, 8*, 623705.

Lowe, A., Harrison, N., & French, A. P. (2017). Hyperspectral image analysis techniques for the detection and classification of the early onset of plant disease and stress. *Plant Methods, 13*(1), 1-12.

Malunga, L. N., Ames, N., Khorshidi, A. S., Thandapilly, S. J., Yan, W., Dyck, A., Waterer, J., Malcolmson, L., Cuthbert, R., Sopiwnyk, E., & Scanlon, M. G. (2021). Association of asparagine concentration in wheat with cultivar, location, fertilizer, and their interaction. *Food Chemistry, 344*, 128630.

Morris, N., Hoad, S., Robertson, D., & Charlton, M. (2022). Project Report No. 642: Nitrogen and sulphur fertiliser management to achieve grain protein quality targets of high-yielding modern winter milling wheat. *Agricultural and Horticultural Development Board.* https://ahdb.org.uk/nitrogen-and-sulphur-fertiliser-management-to-achieve-grain-protein-quality-targets-of-high-yielding-winter-milling-wheat

Oddy, J., Addy, J., Mead, A., Hall, C., Mackay, C., Ashfield, T., McDiarmid, F., Curtis, T.Y., Raffan, S., Wilkinson, M., Elmore, J.S., Cryer, N., Moreira-de-Almeida, I. and Halford, N.G.

(2023). Reducing dietary acrylamide exposure from wheat products through crop management and imaging. *Journal of Agricultural and Food Chemistry, 71*(7), 3403-3413. Doi: 10.1021/acs.jafc.2c07208.

Peivasteh-Roudsari, L., Karami, M., Barzegar-Bafrouei, R., Samiee, S., Karami, H., Tajdar-Oranj, B., Mahdavi, V., Alizadeh, A.M., Sadighara, P., & Khaneghah, A. M. (2023) Toxicity, metabolism, and mitigation strategies of acrylamide: a comprehensive review. *International Journal of Environmental Health Research*. Doi: 10.1080/09603123.2022.2123907

Raffan, S. and Halford, N.G. (2019). Acrylamide in food: progress in and prospects for genetic and agronomic solutions. *Annals of Applied Biology, 175*, 259-281.

Raffan, S., Oddy, J., Mead, A., Barker, G., Curtis, T. Y., Usher, S. L., Burt, C., & Halford, N. G. (2023). Field assessment of genome edited, low asparagine wheat: Europe's first CRISPR wheat field trial. *Plant Biotechnology Journal*. Doi: 10.1111/pbi.14026.

Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., & Tornqvist, M. (2002). Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *Journal of Agricultural and Food Chemistry, 50*(17), 4998-5006.

UK Parliament. (2023). Genetic Technology (Precision Breeding) Bill. https://bills.parliament.uk/bills/3167 (accessed March 30, 2023).

Wilson, T. L., Guttieri, M. J., Nelson, N. O., Fritz, A., & Tilley, M. (2020). Nitrogen and sulfur effects on hard winter wheat quality and asparagine concentration. *Journal of Cereal Science*, *93*, 102969.

Xie, Y., Malunga, L. N., Ames, N. P., Waterer, J., Khorshidi, A. S., & Scanlon, M. G. (2021). Effects of growing environment, genotype, and commercial fertilization levels on free asparagine concentration in Western Canadian wheat. *Cereal Chemistry, 98*(1), 89-99.

FIGURE

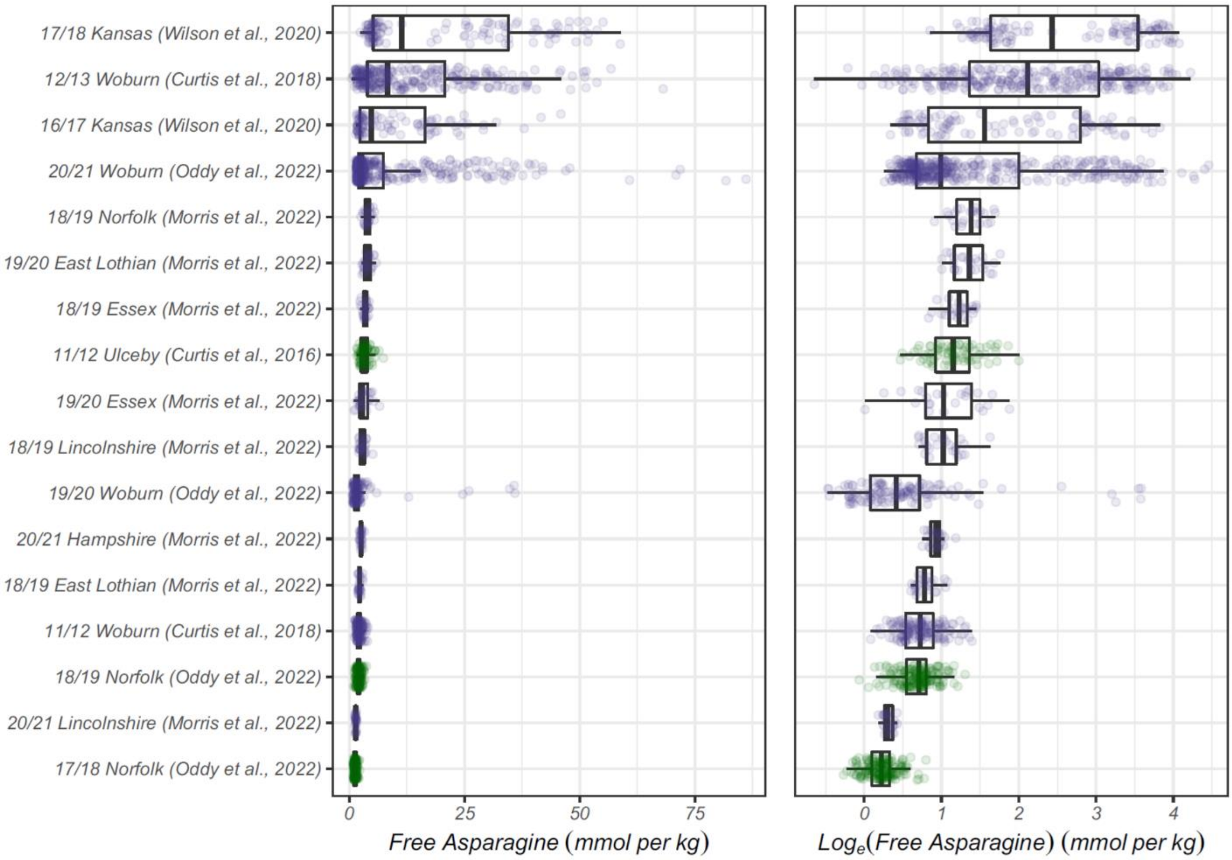

Figure 1. Free asparagine concentrations in wheat grain from 18 different field trials on raw scale (left panel) and log$_e$ transformed (right panel). Trials testing different levels of sulphur are shown in purple and those with consistent levels of fertiliser are shown in green. The trial undertaken by Curtis et al. (2016) contained plots that were either treated or untreated with fungicides. Boxes show the interquartile range and median values, with the whiskers showing the largest or smallest value within 1.5 times below or above the 25th or 75th percentile, respectively.