

Reducing Acrylamide Precursors in Raw Materials Derived from Wheat and Potato

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A review of agronomic and genetic approaches as strategies for the mitigation of acrylamide risk in wheat and potato is presented. Acrylamide is formed through the Maillard reaction during high-temperature cooking, such as frying, roasting, or baking, and the main precursors are free asparagine and reducing sugars. In wheat flour, acrylamide formation is determined by asparagine levels and asparagine accumulation increases dramatically in response to sulfur deprivation and, to a much lesser extent, with nitrogen feeding. In potatoes, in which sugar concentrations are much lower, the relationships between acrylamide and its precursors are more complex. Much attention has been focused on reducing the levels of sugars in potatoes as a means of reducing acrylamide risk. However, the level of asparagine as a proportion of the total free amino acid pool has been shown to be a key parameter, indicating that when sugar levels are limiting, competition between asparagine and the other amino acids for participation in the Maillard reaction determines acrylamide formation. Genetic approaches to reducing acrylamide risk include the identification of cultivars and other germplasm in which free asparagine and/or sugar levels are low and the manipulation of genes involved in sugar and amino acid metabolism and signaling. These approaches are made more difficult by genotype/environment interactions that can result in a genotype being “good” in one environment but “poor” in another. Another important consideration is the effect that any change could have on flavor in the cooked product. Nevertheless, as both wheat and potato are regarded as of relatively high acrylamide risk compared with, for example, maize and rice, it is essential that changes are achieved that mitigate the problem.

KEYWORDS: Acrylamide; asparagine; potato; sugars; agronomy; sulfur; nitrogen; genetic modification; plant breeding

INTRODUCTION

Acrylamide, in its monomeric form, is a neurotoxin and a probable carcinogen. Its presence in cooked foods was first reported in 2002, and concentrations in excess of 1 mg/kg (1000 ppb) have been reported in a variety of potato- and cereal-based products such as crisps, chips (French fries), roasted and baked potatoes, bread, breakfast cereals, and biscuits (1). Acrylamide is formed as a result of the Maillard reaction (2, 3), which involves the thermal degradation of amino acids in the presence of reducing sugars. Although this reaction imparts desirable qualities such as color and flavor to food, in the case of asparagine it also results in the formation of acrylamide. The major precursors for acrylamide formation, therefore, are free asparagine and reducing sugars, although sucrose can participate

in the reaction at very high temperatures because it will undergo decomposition to give reactive carbonyl compounds. Stadler and co-workers reported approximately equal reactivities of fructose, glucose, and sucrose in model reactions with asparagine at a temperature of 180 °C (3), whereas Claeys and co-workers found sucrose to be about 50% as reactive toward asparagine as glucose on a molar basis over temperatures from 140 to 200 °C (4).

International food-monitoring agencies, in collaboration with industry, have put forward strategies for modifying processing methods to reduce dietary acrylamide. These have included prewashes of the raw material, reduction of cooking times and temperatures (5–7), and lowering the pH (5, 8, 9). These methods are described and discussed in more detail elsewhere in these symposium papers and in the “Acrylamide Toolbox” produced by the Confederation of the Food and Drinks Industries of the European Union (CIAA) (www.ciaa.be/documents/brochures/CIAA_Acrylamide_Toolbox_Oct2006.pdf). However, many of these methods limit the extent to which the Maillard

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reaction occurs, and although they result in a reduction of acrylamide, they also compromise the color and flavor of the end-product. Another approach is the topical application of asparaginase to food, converting asparagine to aspartic acid, which has been shown to reduce acrylamide levels in some processed foods (10). This approach may be limited to food matrices that allow for easy take-up and mixing-in of the enzyme.

To date, modifying processing methods has been the main approach used in the mitigation of the acrylamide problem. However, there are anecdotal reports from the food industry that this approach is reaching the limit of what can be achieved and that alternative or complementary strategies need to be developed. Attention is therefore turning to improving the raw material by reducing the levels of sugars and/or free asparagine and thereby the risk of acrylamide formation. Here we review the progress so far and the prospects for this approach.

EFFECTS OF NUTRIENT AVAILABILITY DURING CULTIVATION

Free asparagine accumulation can be induced in many plant species and a variety of organs and tissue types by a multiplicity of stresses, including exposure to toxic metals such as cadmium (11, 12), pathogen attack (13, 14), and drought or salt stress (15, 16) [note that its accumulation under drought and salt stress is often accompanied or exceeded by an accumulation of proline, resulting in its relative contribution to the total amino acid pool actually falling (16)]. It can also be induced in many plant species in response to nutrient availability, with different nutrients having contrasting effects. As a general rule, free asparagine accumulates when the rates of protein synthesis are low and there is a plentiful supply of reduced nitrogen (17). For example, in both cereals and potatoes nitrogen availability correlates positively with both the free asparagine and the total free amino acid content (18, 19). In contrast, when plants are grown with a plentiful supply of nitrogen, deficiencies in potassium, sulfur, phosphorus, or magnesium can cause asparagine levels to rise (20–25).

Sulfur deficiency leads to the greatest asparagine accumulation in cereal grain, and in conditions of severe sulfur deprivation asparagine can accumulate to up to 50% of the total free amino acid pool (26–29). Indeed, Muttucumaru and co-workers (28) demonstrated an up to 30-fold increase in grain asparagine levels in wheat cultivated either in pots or in the field under conditions of severe sulfur deficiency, compared with wheat grown with a plentiful supply of sulfur. This study established for the first time a clear correlation between grain asparagine levels and acrylamide formation in heated wheat flour (28, 29).

Granvogl and co-workers have argued that this is of little relevance to consumers, at least in developed countries, because a farmer would never grow wheat under such poor conditions without applying fertilizer and a baker would not produce bread from low-sulfur grain because the loaf quality would be so poor (30). Indeed, the low yield and poor quality of bread produced from low-sulfur wheat was demonstrated some time ago (31–33). However, we reject this argument and urge that it should not be used as an excuse for complacency. The increase in grain asparagine that occurs when a wheat plant is grown under severe sulfur deprivation is so great that even very small amounts of such grain entering the food chain could have a significant impact on acrylamide risk. For example, in the case reported by Muttucumaru and co-workers (28) in which grain asparagine increased 30-fold in response to sulfur deprivation, if such grain accounted for just 1% of the total harvested, it would increase

Chart 1. Summary of Current Knowledge on the Effect of Agronomic Factors on Acrylamide Risk in Wheat

- Avoiding sulfur, phosphate and potassium deficiency is crucial in reducing acrylamide risk.
- Soil sulfur content should be at least 15 kg per hectare.
- Nitrogen feeding increases acrylamide risk, exacerbating the effects of deficiencies in other minerals. ‘Excess’ nitrogen is ‘stored’ as free asparagine. However, advice has to be realistic: farmers are not going to stop applying nitrogen.
- Other environmental factors, including ‘local’ ones, have a significant impact.
- Advice to growers will have to be tailored for specific areas and soil types.

the total grain asparagine content by 30%. It is entirely conceivable that this could occur, for example, if fertilizer application failed to reach field margins or was applied unevenly, or if an apparently adequate, intrinsic soil sulfur content were distributed unevenly. We are not aware of any studies being carried out to ascertain how often this might occur. Furthermore, although the extreme condition of zero-available sulfur might be unlikely, sulfur feeding at 10 kg/ha may be quite common, and this level available to field-grown wheat caused an increase of approximately 30% in acrylamide formation in heated wheat flour compared with 40 kg of sulfur/ha (28). The minimum recommended level of sulfur for wheat cultivation in the United Kingdom is 15–20 kg/ha, but we have no data on how much wheat is grown at lower levels of sulfur and, indeed, no measurement of acrylamide formation in grain from wheat grown with the recommended 15–20 kg/ha.

The acute response of wheat to sulfur deprivation could be regarded as making wheat “risky” with respect to dietary acrylamide intake. What is more, this risk could be exacerbated by the fact that soil sulfur deficiency is getting worse because of the reduction in atmospheric deposition of sulfur and the switch to nitric acid-based fertilizers in place of sulfur-containing fertilizers such as ammonium sulfate or superphosphate (28, 29, 32). On the other hand, soil sulfur deficiency is a treatable problem, and if sulfur deprivation is responsible for wheat grain having relatively high levels of asparagine, then ensuring that it does not occur represents an opportunity for bringing wheat grain asparagine down to the levels found in other cereals, such as maize and rice. The advice that we can give on wheat cultivation at the present time is summarized in **Chart 1**.

In potatoes the relationship between free asparagine, sugars, and acrylamide is more complicated. Asparagine is the dominant free amino compound, typically accounting for approximately one-third of the total free amino acid pool (34–36). However, as with other plant species, the amount and concentration are influenced by both genetic and environmental factors. Sugars are regarded as limiting with respect to acrylamide formation, but they can accumulate rapidly in tubers stored at 4 °C (the phenomenon of cold sweetening) or in response to other environmental factors (36–38), taking them to and beyond the point at which they are no longer limiting.

Elmore and co-workers (39) performed a study on sulfur feeding of potatoes similar to that which had been performed by Muttucumaru and co-workers on wheat but obtained a very different result. The level of free asparagine did rise in response to sulfur deprivation in one cultivar (Prairie) but nowhere near as dramatically as it did in wheat, and in two cultivars (King Edward and Maris Piper) it declined. More importantly, even

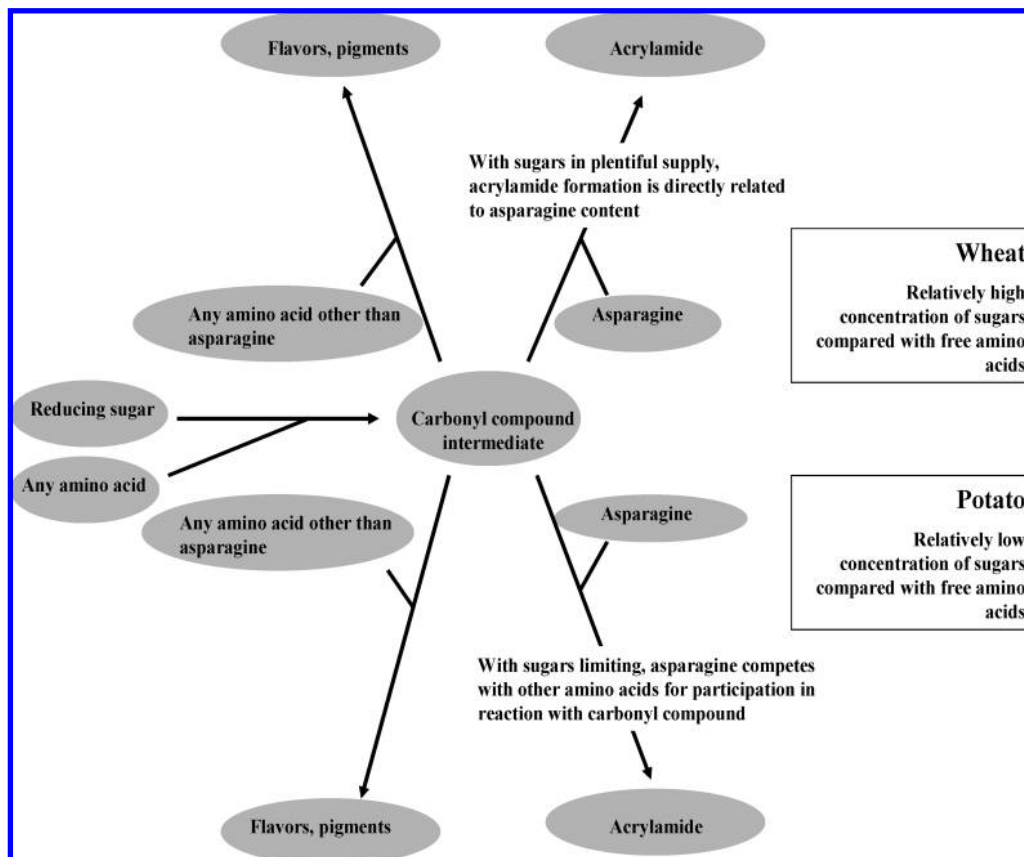


Figure 1. Simple schematic showing the participation of amino acids in the Maillard reaction in wheat, in which free asparagine levels limit acrylamide formation, and in potato, in which sugars are limiting and the ratio of asparagine to the other amino acids in the free amino acid pool is a key parameter.

when there was an increase in asparagine, it was exceeded by an increase in glutamine. This meant that in all three cultivars asparagine levels as a proportion of the total amino acid pool actually fell. Although sugar concentrations increased in some cultivars and decreased in others, the effect of sulfur feeding on acrylamide formation was consistent: increased sulfur feeding led to a rise in acrylamide formation when tuber flour was heated, in direct contrast to the effect of sulfur feeding on wheat. The key parameter here was the concentration of asparagine as a proportion of the total free amino acid pool. We hypothesize that while sugars remained limiting there was competition between asparagine and other amino acids for participation in the Maillard reaction (29, 39) (**Figure 1**), and this determined the amount of acrylamide that was formed.

The significance of this result was that it called into question the dogma that had existed previously that sugar levels were the only parameter that influenced acrylamide formation in potato products (35, 40). It also raised the possibility of exploiting the effect of other amino acids to mitigate acrylamide risk without reducing asparagine or sugar levels. This has significant implications for potato processing because changes in the amino acid and/or sugar profile are likely to affect both the flavor and color of the final product (41).

In summary, we are still some way from being able to give advice to potato farmers with confidence (**Chart 2**).

GENETIC TARGETS

The most direct genetic approach to reducing acrylamide risk is to exploit the differences between genotypes that already exist. For example, Taeymans and co-workers (42) reported that the free asparagine content of different European wheat varieties

harvested in 2002 ranged from 163 to 664 mg/kg of dry weight. They also reported large differences between samples of the same variety, indicating that environmental factors were having a significant effect. However, Claus and co-workers (43) also reported large differences in flour asparagine content for different wheat varieties, from 49 mg/kg (Terrier) to 249 mg/kg (Transit). This correlated with acrylamide content in heated flour ranging from 154 to 624 $\mu\text{g}/\text{kg}$.

Several groups have reported that the free asparagine content of tubers from different potato cultivars varies, although within a narrower range than that reported for wheat (44–46). In potato, as we have discussed above, sugars have also received much attention, and Amrein and co-workers (35), for example, reported that 17 potato cultivars grown in Switzerland in 2002 showed large differences in their potential for acrylamide formation and that this was primarily related to their sugar contents. Other authors have reported greater differences for purchased tubers of different varieties, but it is impossible to assess whether this was due to genotype or other factors because the potatoes were purchased from the retail sector, with no knowledge of the duration or temperature of their storage.

These studies indicate that there is scope for using varietal differences in asparagine and sugars to address the acrylamide issue, in both wheat and potato. However, we are not aware of large-scale programs aimed at doing this. Indeed, the anecdotal reports that have come to us indicate that the acrylamide issue appears to have exposed a lack of communication between food processors and their suppliers.

Longer-term attempts to reduce the levels of acrylamide precursors by breeding will focus on the genes of sugar and amino acid metabolism and the signaling networks that control

Chart 2. Summary of Current Knowledge on the Effect of Agronomic Factors on Acrylamide Risk in Potato

- Both sulfur and nitrogen feeding increase acrylamide risk.
- The relationship between amino acids, sugars and acrylamide is complex.
- The change in amino acid profile in response to feeding is highly variety-dependent.
- Storage at 4 °C must be avoided because it causes a rapid accumulation of sugars and a concomitant increase in acrylamide risk.
- Advice to growers will have to be tailored for specific areas, varieties and soil types.

them. Wheat grain and potato tubers are carbon sinks and are supplied with carbon from the leaves in the form of sucrose. Plants have two enzymes that can cleave sucrose: invertase and sucrose synthase. The reaction catalyzed by invertase produces glucose and fructose, principally for flux into glycolysis (47). The reaction catalyzed by sucrose synthase requires uridine diphosphate (UDP) and produces UDP-glucose and fructose (under physiological conditions the equilibrium of this reaction very much favors cleavage over synthesis, despite the name of the enzyme). In potato tubers it is involved principally in supplying the starch biosynthetic pathway (48). Sugars can also be produced from the breakdown of storage compounds, of which the major one in wheat and potato is starch. Rommens and co-workers (49) showed the potential of targeting this area of carbohydrate metabolism in potato by using RNA inhibition techniques to reduce the expression of the starch-associated *RI* and phosphorylase-L (*PhL*) genes. The enzymes encoded by these genes are both involved in the degradation of starch during cold storage. Glucose levels in the transgenic lines were up to 80% lower than in the controls after 3 months of storage at 4 °C, and acrylamide levels after processing were <0.2 mg/kg (200 ppb) in one transgenic line compared with almost 1.2 mg/kg (1200 ppb) in unmodified controls.

Progress has been made in elucidating the signaling networks that regulate carbon metabolism (50, 51), and overexpression of a key metabolic regulator, sucrose nonfermenting-1-related protein kinase-1 (SnRK1) has been shown to result in an

increase in starch and a decrease of up to 80% in glucose concentration (52). However, in this case the effect on acrylamide formation has not yet been reported.

The enzymes involved in the biosynthesis and degradation of asparagine are shown in **Figure 2**. Biosynthesis requires the assimilation of ammonia to produce glutamine, a reaction mediated by the enzymes glutamine synthetase and glutamate synthase (17, 53, 54); the glutamine then provides the nitrogen required for the formation of asparagine from aspartate, catalyzed by the enzyme asparagine synthetase. The same enzyme catalyzes the adenosine triphosphate-dependent reaction between glutamine and aspartate to generate glutamate. Two other key enzymes are asparaginase, which catalyzes the hydrolysis of asparagine resulting in the generation of aspartate and ammonia, and aspartate kinase, which competes for aspartate to produce 4-aspartyl phosphate, a precursor for the amino acids methionine, threonine, lysine, and isoleucine.

The genes that encode these enzymes are all potential candidate genes either for manipulation by genetic modification or mutagenesis, or for the development of genetic markers for breeding programs.

CONCLUDING REMARKS

Acrylamide was first discovered in foods in 2002 (55); at that time and ever since, consumers have appeared to remain relatively calm about the issue, in quite dramatic contrast to, for example, the attitude of European consumers to genetically modified foods or the effect on the beef market of the spread of bovine spongiform encephalopathy in the U.K. cattle herd in the latter part of the last century. Even the first publication of an epidemiological study linking dietary intake of acrylamide with increased cancer risk in humans (56) did not cause widespread alarm. Epidemiological studies are notoriously unreliable, and mainstream reporting of the study was confused and ill-informed; the British Broadcasting Corporation, for example, first reported that the problem was linked to fatty foods and then to burnt foods (<http://news.bbc.co.uk/1/hi/health/7124501.stm>). Nevertheless, the lack of response from consumers was remarkable.

Despite this, the acrylamide issue remains a threat to the sustainability of the wheat and potato production and processing industries and cannot be ignored. Further evidence of a link

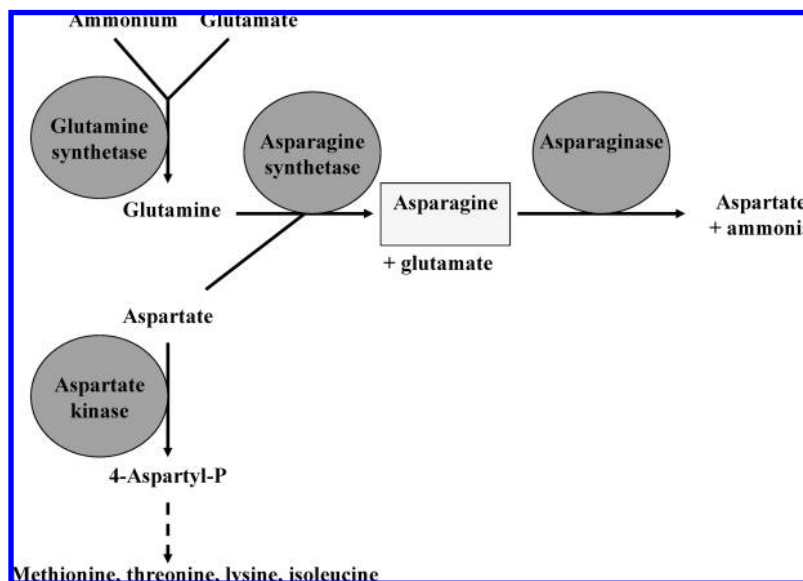


Figure 2. Scheme showing the enzymes involved in asparagine synthesis and breakdown.

between dietary intake of acrylamide and cancer in humans could provoke a stronger response from consumers; it could also force regulators to set statutory limits for acrylamide levels and increase pressure on the food industry to reduce levels further. A possible solution for the food industry if that were to occur would be to replace relatively high-risk raw materials, such as wheat and potato, either completely or partly with lower risk raw materials. It is probably no coincidence that since 2002 some popular potato- and wheat-based snacks have been joined on the supermarket shelves by rice- and maize-based alternatives.

Considerably more work needs to be done before firm advice can be given to farmers and plant breeders on the best ways forward. Whereas low asparagine appears to be an obvious target for wheat, the situation in potato is more complex, with sugar levels and competition between asparagine and other amino acids being the key parameters. In either case, more understanding is required of the effect of the amino acid profile and sugar level on color and flavor development and how this can be balanced against reducing acrylamide formation.

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