



Genetic and agronomic approaches to decreasing acrylamide precursors in crop plants

Nigel G. Halford, Nira Muttucumaru, Tanya Y. Curtis, Martin A.J. Parry

► To cite this version:

Nigel G. Halford, Nira Muttucumaru, Tanya Y. Curtis, Martin A.J. Parry. Genetic and agronomic approaches to decreasing acrylamide precursors in crop plants. Food Additives and Contaminants, 2007, 24 (S1), pp.26-36. 10.1080/02652030701403093 . hal-00577461

HAL Id: hal-00577461

<https://hal.science/hal-00577461>

Submitted on 17 Mar 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Genetic and agronomic approaches to decreasing acrylamide precursors in crop plants

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2007-043.R1
Manuscript Type:	Review
Date Submitted by the Author:	12-Apr-2007
Complete List of Authors:	Halford, Nigel; Rothamsted Research, Crop Performance and Improvement Division Muttucumaru, Nira; Rothamsted Research, Crop Performance and Improvement Curtis, Tanya; Rothamsted Research, Crop Performance and Improvement Parry, Martin; Rothamsted Research, Crop Performance and Improvement
Methods/Techniques:	GC/MS
Additives/Contaminants:	Acrylamide
Food Types:	Cereals and grain, Cooked foods, Plants, Potatoes

SCHOLARONE™
Manuscripts

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Genetic and agronomic approaches to decreasing acrylamide precursors in crop plants

Nigel G. Halford*, Nira Muttucumaru, Tanya Y. Curtis and Martin A.J Parry

Centre for Crop Genetic Improvement, Plant Sciences Department, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, United Kingdom.

*Author for correspondence: Telephone 44 1582 763133; Fax 44 1582 763010; Email nigel.halford@bbsrc.ac.uk

Review for Special Issue: ‘Update on the Progress in Acrylamide and Furan Research’

Abstract

Progress in developing genetic and agronomic approaches for reducing the levels of the principal precursors of acrylamide, asparagine and sugars, in crop plants is reviewed. The factors that affect asparagine and sugar accumulation, particularly in cereal seeds and potato tubers, are described. Asparagine levels appear to be the key parameter in determining acrylamide formation in processed wheat flour and agronomic strategies for reducing asparagine accumulation in wheat grain are reviewed. Sulphur availability has been shown to be particularly important, with sulphur deprivation causing a dramatic increase in grain asparagine levels and acrylamide risk. Nitrogen availability is also a factor, with increasing nitrogen availability causing grain asparagine levels and acrylamide risk to rise. In potato, attention has been focussed on sugars, and there has been some success in reducing sugar accumulation in stored potatoes by genetic modification, with a resultant reduction in acrylamide formation. However, the wisdom or otherwise of this dogma is discussed. Other possible genetic targets for manipulation or development as genetic markers in breeding programmes are reviewed. Plant breeders and farmers are encouraged to exploit the varietal differences in acrylamide risk that have already been identified and to develop good agronomic practice to reduce the levels of acrylamide precursors in cereals and potato.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Introduction

Foods with the highest levels of acrylamide are carbohydrate-rich and include those derived from potato, wheat, maize and other cereals, as well as coffee. A number of proposals have been put forward to lower acrylamide levels by changing processing methods; they include reducing cooking times and temperatures (Amrein *et al.*, 2004, Surdyk *et al.*, 2004, Taubert *et al.*, 2004) and lowering the pH (Amrein *et al.*, 2004, Jung *et al.*, 2003, Rydberg *et al.*, 2003). However, the Maillard reaction, which is responsible for acrylamide formation, also generates desirable flavour and colour compounds in heated food, so reducing the cooking time and temperature, or lowering the pH may reduce colour and flavour development. There must, therefore, be a limit to what can be achieved by these methods. The use of asparaginase to convert asparagine to aspartic acid has been successful in reducing acrylamide formation in some foodstuffs (Zyzak *et al.*, 2003) but it has not yet been approved for commercial use or shown to be practical or cost-effective in large-scale food production and is only likely to work in those foods that allow take-up and mixing-in of the enzyme.

The use of raw materials with low sugar or asparagine content is an alternative or complementary solution and this makes the problem of acrylamide in food a plant and agronomic sciences issue as well as a food processing one. This article reviews progress in this area and the prospects for developing new varieties and agronomic strategies that help to meet the challenge of reducing dietary intake of acrylamide. First we must consider the factors that affect the accumulation of the principal precursors for acrylamide, asparagine and sugars, in plants.

Factors controlling asparagine accumulation

Asparagine accumulates in all plant tissues, but in the context of acrylamide formation the asparagine content of the edible parts of plants (e.g. seeds and tubers) is of greatest

1
2
3 relevance. In potato tubers, asparagine is the dominant free amino compound, typically
4
5 accounting for approximately one third of the total free amino acid pool (Eppendorfer and
6
7 Bille, 1996). In contrast, in cereal grains asparagine usually accounts for less than 10 % of
8
9 the total free amino acids (Lea *et al.*, 2007). However, the amount and concentration is
10
11 influenced by both genetic and environmental factors. In general, asparagine accumulates
12
13 when the rates of protein synthesis are low and there is a plentiful supply of reduced nitrogen.
14
15 Whilst asparagine accumulates during normal physiological processes, such as seed
16
17 germination, its accumulation can also be induced by both biotic and abiotic stresses. This
18
19 can occur either as a result of inhibition of protein synthesis or by direct effects on asparagine
20
21 metabolism.
22
23
24
25
26
27
28

29 *Nutrient Supply*

31
32 In both cereals and potatoes nitrogen availability correlates positively with both the
33
34 free asparagine and the total free amino acid content, thus asparagine concentration increases
35
36 but its contribution to the total free amino acid pool remains relatively static (De Wilde *et al.*
37
38 2006, Winkler and Schön, 1980). When plants are grown with a plentiful supply of nitrogen,
39
40 deficiencies in potassium, sulphur, phosphorus and magnesium, but not molybdenum,
41
42 stimulate accumulation of asparagine (Possingham, 1956, Stewart and Larher, 1980, Rabe and
43
44 Lovatt, 1986, Rufty *et al.*, 1990, Almeida *et al.*, 2000, Nikiforova *et al.*, 2006). However
45
46 sulphur deficiency leads to the greatest asparagine accumulation and in conditions of severe
47
48 sulphur deprivation asparagine can accumulate to up to 50 % of the total free amino acid pool
49
50 (Shewry *et al.*, 1983, Baudet *et al.*, 1986, Muttucumaru *et al.*, 2006). This is discussed in
51
52 more detail later in the review.
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Toxic metals

Exposure to toxic metals can cause accumulation of some amino acids, including asparagine. For example, cadmium, a major pollutant worldwide, has been shown to induce asparagine accumulation in both lettuce and lupins (Costa and Morel, 1994, Costa and Spitz, 1997). Asparagine can bind to or react with cadmium, copper, lead and zinc to generate less toxic compounds (White *et al.* 1981, Bottari and Festa, 1996, Smirnoff and Stewart, 1987)

Pathogen attack

Fungal and bacterial attack can lead to very large increases in asparagine accumulation. For example, an 800-fold increase in asparagine concentration has been reported in tomato leaves infected with the bacteria *Pseudomonas syringea* (Perez-Garcia *et al.*, 1998) and a similarly large increase has been reported in cocoa infected with the basidiomycete fungus *Crinipellis pernicioso* (Scarpari *et al.*, 2005).

Drought and salt stress

Asparagine shows a characteristic linear increase in plants subjected to drought stress. However, asparagine as a percentage of the total free amino acids may fall as proline often accumulates to a much greater extent (Good and Zaplachinski, 1994). In most species, accumulation of asparagine and proline under salt stress is similar to that under drought (Garthwaite *et al.*, 2005).

Factors controlling sugar accumulation

Carbon is, of course, derived by plants from atmospheric CO₂ by photosynthesis and generally sugar availability is dependent upon that process. The major harvested organs of crop plants, such as seeds and tubers, however, are not themselves photosynthetic; they are

1
2
3 carbon sinks that derive all of their carbon from the leaves in the form of sucrose. The first
4
5 effect of a change in the supply of sucrose to the developing seed or tuber is probably one of
6
7 yield, rather than of concentration, so we would predict that it would have a relatively small
8
9 effect on acrylamide formation, although there is little data to support this hypothesis. The
10
11 principal determinant of acrylamide formation will be what happens to the sucrose when it
12
13 arrives at the sink organ, in other words how it is partitioned between different end products
14
15 and intermediates, including reducing sugars such as glucose, fructose and maltose that can
16
17 participate in the Maillard reaction.
18
19
20
21

22 A large proportion of the sucrose that is imported into a potato tuber or cereal
23
24 seed is incorporated into starch, oils or storage proteins and stored. On seed germination or
25
26 tuber sprouting these storage compounds are remobilised and sugars are released for use in
27
28 providing energy and the raw materials for growth. Prevention of sprouting during storage
29
30 and the exclusion of sprouted material from the food chain may therefore be important in
31
32 reducing acrylamide risk, depending on the type of food that the material is destined for and
33
34 the nature of the processing that it will undergo.
35
36
37
38

39 A complicating factor in the subject of sugar metabolism in plants is the fact that
40
41 sugars themselves are key signalling metabolites and the signals that they initiate have
42
43 profound effects on enzyme activity and gene expression, thereby influencing the flux of
44
45 compounds through metabolic pathways. In this way, sugar levels affect and are affected by
46
47 processes such as photosynthesis, the cell cycle, biotic and abiotic stresses from the
48
49 environment, nutrient availability and the partitioning of resources within the plant. This
50
51 subject is reviewed elsewhere (Halford and Paul, 2003, Halford, 2006) and we will not review
52
53 it further here, but it must be remembered that changing sugar levels can have profound and
54
55 wide-ranging effects on primary and secondary metabolism.
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Agronomic strategies for reducing the levels of acrylamide precursors

Effects of sulphur availability

Sulphur (S) is an essential macronutrient required by plants for growth. Inorganic sulphate is acquired from the soil and uptake is mediated by specialised sulphate transporters at the root-soil interface. It is subsequently assimilated into the amino acids cysteine and methionine and a variety of other metabolites.

Ironically, sulphur nutrition of crops benefited in the past from an otherwise undesirable by-product of the industrial revolution, namely atmospheric SO₂, generated predominately from the burning of fossil fuels. While being environmentally damaging in other ways, the deposition of this atmospheric sulphur in the form of ‘acid rain’ helped to maintain the sulphur content of soils used for crop production. ‘Old-fashioned’ fertilizers such as ammonium sulphate and superphosphate (figure 1) also contained sulphur, although they were marketed principally as sources of nitrogen and phosphate. This meant that sulphur nutrition was of little practical concern in the UK. However, in the latter part of the last century there was a huge switch from the burning of coal as a fuel to low sulphur or sulphur-free alternatives such as natural gas. At the same time, fertilizers such as ammonium sulphate and superphosphate had been replaced by fertilisers based on nitric acid, better sources of nitrogen but containing no sulphur, and increasing crop yields were resulting in a more rapid removal of soil minerals, including sulphur. As a result, by the end of the century increasing areas of land were beginning to become sulphur deficient (Zhao *et al.* 1999) and the Home Grown Cereals Authority (www.hgca.com) estimates that 23 % of the United Kingdom is now at high risk from sulphur deficiency for cereal cultivation. Sulphur deficiency has also been reported in Northern and Western Europe, Australia and New Zealand (Zhao *et al.*, 2002, Blair, 2002).

The relevance of all of this to the acrylamide issue arises from the effect of sulphur deprivation on asparagine accumulation. A study undertaken in 1983 demonstrated that grain harvested from barley grown under conditions of sulphur deprivation contained a reduced amount of total protein and a high content of aspartic acid and asparagine (Shewry *et al.*, 1983, 2001). More recently, Muttucumaru and co-workers reported that wheat grown under conditions of severe sulphate depletion showed dramatic increases in the concentration of free asparagine in the grain of up to thirty times compared with samples receiving the normal levels of sulphate fertilizer. The effect was observed both in plants grown in pots (figure 2), where the levels of nutrients were carefully controlled, and in plants grown in field trials on soil with poor levels of natural nutrients (figure 3). When flours from the sulphate-deprived wheat were heated at 160 °C for 20 minutes levels of acrylamide between 2600 and 5200 µg per kg were found compared with 600 to 900 µg per kg in wheat grown with normal levels of sulphur fertilization.

It is unlikely that any wheat would be grown in the UK or other developed country under conditions of complete sulphur deprivation without a farmer intervening because the effect on the growth of the plants and grain yield would be so obvious (figure 4). However, it is possible that plants growing around the margins of fields where sulphur application is required due to poor soil nutrient retention could be severely sulphur deprived if fertilizer application did not reach them. The entry of grain from such plants into the food chain might explain why food processors have made anecdotal reports of unpredictable spikes of acrylamide formation affecting some batches of their products. Furthermore, the results of Muttucumaru and co-workers showed that even flours from wheat grown in the field with an intermediate level of sulphur application (10 kg sulphur per hectare) contain more acrylamide after processing than flours from wheat grown with an application of 40 kg sulphur per hectare (figure 3) (Muttucumaru *et al.*, 2006). Perhaps significantly, the higher level of

acrylamide was above the 1000 ppb mark that has been used as a *de facto* limit for acrylamide content by German regulatory authorities and which could be adopted in other countries should they decide to follow Germany's lead (Germany is the only country to have set such a limit to date).

Clearly, the importance of maintaining sulphur levels for cereal cultivation must be stressed to farmers. However, it should be noted that while the effect of sulphur deprivation on asparagine accumulation appears to be widespread in plants, its link with acrylamide formation has not been demonstrated for other types of crop plants. In potato, for example, while sulphur deprivation is to be avoided because of its effect on plant development and yield, which is as severe as it is for wheat (figure 4), there are indications that the relationship between sulphur feeding and acrylamide formation in processed tuber material is more complicated than it is for wheat grain (unpublished data). It would therefore be premature to issue advice to potato farmers concerning sulphur feeding and the acceptability of their product with respect to acrylamide formation.

Effects of nitrogen availability

In a recently published study it was reported that the application of nitrogen (N) fertiliser to wheat resulted in an increase in the levels of free amino acids and protein content in the grain (Claus *et al.*, 2006). Neither of these is a novel finding (see Shewry *et al.*, 2001, for a review of work in this area) but the study of Claus and co-workers showed that the increase in amino acid levels resulted in an increase in acrylamide formation in the flour after heating. A simple schematic of the steps in the Maillard reaction is shown in figure 5 and reminds us that amino acids are involved both in the formation of the carbonyl compound intermediates and in the conversion of those intermediates to flavour and colour compounds or, in the case of asparagine, acrylamide (Mottram *et al.*, 2002, Stadler *et al.*, 2002, Wedzicha

1
2
3 *et al.*, 2005). An increase in any amino acid can therefore drive the reaction by participating
4
5 in the first step and this as well as an increase in asparagine itself is the likely explanation for
6
7 the effect of nitrogen on acrylamide formation.
8
9

10 The fact that increasing nitrogen supply also increases protein content is important
11
12 because clearly this will mop up some of the free amino acids. However, this will not occur if
13
14 sulphur is in short supply because sulphur deprivation results in a decrease in the synthesis of
15
16 the major seed storage proteins (Shewry *et al.*, 1983, 2001). The effects of an increase in
17
18 nitrogen supply are therefore likely to be exacerbated by sulphur deprivation, and *vice versa*.
19
20
21

22 The effect of increasing nitrogen supply is relatively small compared with that of
23
24 sulphur deprivation. Furthermore, while incorporating sulphur into their fertilizer
25
26 applications will no doubt incur some cost to the farmer, that cost might be offset somewhat
27
28 by an increase in the yield and/or quality of the product. In contrast, reducing the application
29
30 of nitrogen will give an initial saving in fertilizer costs but this will be more than offset by a
31
32 reduction in yield and/or quality. There are other reasons for reducing the use of nitrogen
33
34 fertilizer, of course, notably the impact that nitrogen run-off has on water quality. However,
35
36 the issue is a controversial one and farmers are likely to be reluctant to reduce nitrogen
37
38 application.
39
40
41
42

43 Two studies have been published concerning the effect of nitrogen on potato: De
44
45 Wilde and co-workers (2006) studied how potatoes were affected by nitrogen levels in the soil
46
47 and found that levels of reducing sugars rose in nitrogen-deprived potatoes by up to 100 %,
48
49 resulting in an increase in acrylamide in French fries of up to 65 % compared with adequately
50
51 fertilized potatoes. On the other hand, Amrein and co-workers (2003) found no correlation
52
53 between the amount of nitrogen fertilization and acrylamide formation.
54
55
56
57
58
59
60

Storage and sprouting

Potato tubers have to be stored for long periods to enable a year-round supply for consumers and the food industry. The tubers are chilled to prevent sprouting but storage at 4 °C, which is very effective at preventing sprouting, results in the phenomenon of cold sweetening in which starch is broken down and reducing sugars accumulate. This difficult problem predates the acrylamide issue because, as well as being precursors for acrylamide, sugars cause blackening during frying. It is also complicated by the end-use of the potatoes in that potatoes that are produced specifically for crisp production are allowed to be sprayed at harvest with chemical sprout suppressants such as chloropropham (CIPC). This enables the potatoes to be stored at 12 °C, a temperature high enough for cold sweetening not to occur. There are concerns regarding residues of these chemicals in the food product (Food Standards Agency, 2006) and the maximum residue limit (MRL) for CIPC has been reduced recently to 10 ppm. However, the practice is allowed for crisping potatoes because the potatoes are always peeled before use and the MRL is not exceeded. Not so for the fresh potato market and for that reason the use of chemical sprouting suppressants on potatoes destined for retail is not allowed; a balance therefore has to be achieved between sprouting control and the prevention of cold sweetening. Clearly, the discovery that sugars are precursors for acrylamide formation makes this already intractable problem all the more important.

Sprouting is also a major factor for acrylamide formation in cereal grain, as it leads to degradation of starch and proteins, and the release of sugars and asparagine. Claus and co-workers (2006) reported a 500 % increase in acrylamide formation, from 54.5 ppb to 273.3 ppb, in bread prepared from sprouted grain compared with non-sprouted grain and recommended that sprouted grain should not be used for bakery products. The implications of this for the malting and brewing industries were not investigated but high levels of acrylamide have not been reported for beers or spirits.

Other agronomic targets

Theoretically, any of the environmental factors described in the previous sections on asparagine and sugar accumulation that are under human control could be the target of changes in agronomic practice to reduce acrylamide formation. However, while it is likely that maintaining potassium, phosphorus and magnesium availability would help to avoid increases in asparagine accumulation there is little or no information available on how significant or cost-effective this would be. Clearly, some environmental factors, such as rainfall, are out of human control. A possible but controversial strategy would be for food processors to avoid using material produced under environmental conditions, such as drought, if the increase in asparagine content brought about by growth in those conditions showed a clear link with acrylamide risk.

Genetic targets

Varietal differences

Several groups have reported that the asparagine content of tubers from different potato cultivars grown at the same site vary within a narrow range (De Wilde *et al.*, 2005; Matsuura-Endo *et al.*, 2006; Olsson *et al.*, 2004). Although other authors have reported much greater (almost 50 fold) differences for purchased tubers of different varieties it is impossible to assess whether this is due to genotype or other factors. Indeed, a frustration in reviewing this data is that many of the studies have relied on the analysis of potatoes purchased from the retail sector, with no knowledge of the time that the tubers have been in storage or the conditions in which they have been kept. Rigorous, systematic studies are therefore still required to determine genotype differences in asparagine and free amino acid content. Plant

breeding and food processing companies may have undertaken such studies themselves but there is little hard data in the public domain.

Much attention is now being focussed on the sugar content of different potato varieties. Amrein and co-workers (2003) reported that seventeen 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. It was concluded that acrylamide formation in potato products could be reduced substantially by selecting cultivars with low concentrations of reducing sugars. However, while we agree that sugar levels are a key parameter we believe that the relationship between sugars, asparagine and other amino acids in the determination of acrylamide risk in potato products is more complicated than this study indicated, and that the advice to concentrate solely on sugar levels is premature.

In contrast, the advice that asparagine content is the key parameter in cereal grain appears to be sound and significant differences between varieties have been reported. For example, Claus and co-workers (2006) reported an approximately five-fold difference in flour asparagine content for different varieties, from 4.90 mg per 100g (Terrier) to 24.9 mg per 100g (Transit). This correlated with an acrylamide content in heated flour ranging from 153.7 µg per kg to 624.1 µg per kg. Similarly, Taeymans and co-workers (2004) reported a study of the asparagine content of different European wheat varieties harvested in 2002. The level ranged from 163 to 664 mg per kg. However, large differences between samples of the same cultivar indicated that environmental factors were also having a significant effect.

Clearly, a reduction in acrylamide risk in wheat products could be achieved already, simply by growing cultivars that do not accumulate very high levels of asparagine in the grain. The wide variation in asparagine content also suggests that this key parameter should be amenable to plant breeding.

Genes involved in asparagine biosynthesis and amino acid signalling

Biosynthesis of asparagine, as shown in figure 6, involves the initial assimilation of ammonia to produce glutamine, a reaction mediated by the enzymes glutamine synthetase and glutamate synthase (Lea and Miflin, 2003). Glutamine then provides the nitrogen required for asparagine biosynthesis, a reaction which is catalysed by the enzyme asparagine synthetase. Asparagine synthetase also catalyses the adenosine triphosphate-dependent reaction between glutamine and aspartate to generate glutamate. Another key enzyme involved in asparagine metabolism is asparaginase: this enzyme catalyses the hydrolysis of asparagine, resulting in the generation of aspartate and ammonia. Clearly, glutamine synthetase, asparagine synthetase and asparaginase are all potential candidate genes either for manipulation by genetic modification or mutagenesis, or for the development of genetic markers for breeding programmes.

Genes involved in amino acid signalling could also be useful targets for manipulation. One such gene is general control nonderepressible-2 (GCN2), a protein kinase known to be involved in both the control of protein synthesis and the induction of expression of amino acid biosynthesis genes (Zhang *et al.*, 2003).

Genes involved in sugar metabolism

Plants have two enzymes that can cleave sucrose; these are invertase, which cleaves the sucrose molecule to produce glucose and fructose, and sucrose synthase, which in the presence of UDP cleaves sucrose to form UDP-glucose and fructose (note that under physiological conditions the equilibrium of this reversible reaction very much favours cleavage over synthesis, despite the name of the enzyme). In potato tubers, sucrose synthase is the predominant enzyme for sucrose metabolism (Morrell and Ap Rees, 1986) and appears to be involved in supplying the starch biosynthetic pathway (figure 7), while invertase is

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

principally involved in supplying hexoses for flux into glycolysis. Hence, over-expression of invertase together with glucokinase results in a dramatic reduction in starch accumulation and an increase in glucose and glucose phosphates (Trethewey *et al.*, 1998). In contrast, up-regulation of sucrose synthase gene expression together with ADP-glucose pyrophosphorylase, another enzyme in the starch biosynthetic pathway, results in an increase in starch and a decrease of up to 80 % in glucose concentration (McKibbin *et al.*, 2006). The latter was achieved by over-expressing a metabolic regulator, SNF1-related protein kinase-1 (SnRK1), which controls the flux of carbon into the starch biosynthetic pathway in potato tubers (figure 7).

One word of caution concerning sugar levels in potatoes as a target is that the major crisp and French fry manufacturers already use very low sugar varieties but still have a significant acrylamide problem. Furthermore, there must be a sugar level below which colour and flavour formation is not acceptable and if significant levels of acrylamide are still formed then other strategies will have to be adopted. However, the development of varieties that can be stored at 4 °C without accumulating sugars is a sensible target and success in this area has already been reported by Rommens and co-workers (2006), who used RNA inhibition techniques to reduce the expression of the starch-associated *R1* and phosphorylase-L (*PhL*) genes in variety Russet Ranger. 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 three months of storage at 4 °C and acrylamide levels after processing were less than 200 ppb in one transgenic line compared with almost 1200 ppb in unmodified Russet Ranger controls.

This result appears to be a clear indication that a genetic approach to reducing acrylamide risk is a viable strategy and one worth pursuing.

Conclusions

Acrylamide is widely used in industry; it is also familiar to biochemists as a constituent of gels used for the electrophoretic separation of protein, DNA and RNA molecules. Its familiarity probably made the discovery that it is present in many cooked foods all the more shocking for the science community, the food industry and regulatory authorities. Reassurance can be derived from the fact that the presence of acrylamide in foods does not represent a new risk to consumers. Furthermore, its discovery prompted an immediate reaction from the food industry to modify their processes where possible to reduce its formation. Food safety is therefore being improved, an example of how science and technology can benefit consumers. Nevertheless, the levels of acrylamide in foods such as French-fried potatoes, breakfast cereals, cereal- and potato-based snacks and crispbreads are still commonly several hundred ppb.

Estimates of the intake levels of acrylamide from the diet are much lower than the levels that are known to cause toxicological effects in rodents and to date no epidemiological study has found a link between dietary exposure to acrylamide and cancer risk (Mucci *et al.*, 2003, Pelucchi *et al.*, 2003). The position taken by most regulatory authorities to date, therefore, which has been to state that they wish to see dietary intake of acrylamide reduced rather than to set limits for the presence of acrylamide, would appear to be a sensible one. Nonetheless, regulatory authorities may feel obliged to take stronger action if consumer and/or scientific concern increases and plant breeders and farmers should be preparing for such an eventuality. Unfortunately, there is little indication that they are.

We have already identified one agronomic factor, sulphur nutrition, that has a clear, unambiguous effect on acrylamide risk in cereals and the importance of avoiding sulphate deficiency should be made clear to farmers. It is also evident that considerable reductions in acrylamide risk could be achieved simply by variety selection, in both potato and cereals.

More research is required before advice is given on other agronomic and genetic targets, but there is enough evidence available already to justify the conclusion that genetic and agronomic approaches could play a significant part in mitigating the acrylamide problem.

Acknowledgements

Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council (BBSRC) of the United Kingdom. Nira Muttucumaru is financially supported by the BBSRC in conjunction with the United Kingdom's Food Standards Agency. Tanya Curtis is financially supported by the Home Grown Cereals Authority of the United Kingdom.

References

Almeida J. P .F., Hartwig U. A., Frehner M., Nösberger J., and Lüscher A., 2000, Evidence that P deficiency induces nitrogen feedback regulation of symbiotic nitrogen fixation in white clover (*Trifolium repens* L.). *Journal of Experimental Botany*, 51, 1289-1297.

Amrein, T. M., Bachmann, S., Noti, A., Biedermann, M., Barbosa, M. F., Biedermann-Brem, S., Grob, K., Keiser, A., Realini, P., Escher, F. and Amadò, R., 2003, Potential of acrylamide formation, sugars, and free asparagine in potatoes: A comparison of cultivars and farming systems. *Journal of Agriculture and Food Chemistry*, 51, 5556-5560.

Amrein, T. M., Schönbächler, B., Rohner, F., Lukac, H., Schneider, H., Keiser, A., Escher, F. and Amadò, R., 2004, Potential for acrylamide formation in potatoes: data from the 2003 harvest. *European Food Research and Technology*, 219, 572-578.

- Baudet, J., Huet, J-C., Jolivet, E., Lesaint, C., Mossé, J. and Pernollet, J-C., 1986, Changes in accumulation of seed nitrogen compounds in maize under conditions of sulphur deficiency. *Physiologia Plantarum*, 68, 608-614.
- Blair, G. J., 2002, Sulphur fertilisers: A global perspective. International Fertiliser Society Proceeding No 498 (York: The International Fertiliser Society).
- Bottari, F. and Festa M. R., 1996, Asparagine as a ligand for cadmium (II), lead (II) and zinc (II). *Chemical Speciation and Bioavailability*, 8, 75-83.
- Claus, A., Schreiter, P., Weber, A., Graeff, S., Herrmann, W., Claupein, W., Schieber, A. and Carle, R., 2006, Influence of agronomic factors and extraction rate on the acrylamide contents in yeast-leavened breads. *Journal of Agriculture and Food Chemistry*, 54, 8968-8976.
- Costa, G. and Morel, J. L., 1994, Water relations, gas exchange and amino acid content in Cd-treated lettuce. *Plant Physiology and Biochemistry*, 32, 561-570.
- Costa, G. and Spitz, R., 1997, Influence of cadmium on soluble carbohydrates, free amino acids, protein content of *in vitro* cultured *Lupinus albus*. *Plant Science*, 128, 131-140.
- De Wilde, T., De Meulenaer, B., Mestdagh, F., Govaert, Y., Vandeburie, S., Ooghe, W., Fraselle, S., Demeulemeester, K., Van Peteghem, C., Calus, A., Degroot, J. M. and Verhe, R.A., 2005, Influence of storage practices on acrylamide formation during potato frying. *Journal of Agricultural and Food Chemistry*, 53, 6550-6557.
- De Wilde, T., De Meulenaer, B., Mestdagh, F., Govaert, Y., Vandeburie, S., Ooghe, W., Fraselle, S., Demeulemeester, K., Van Peteghem, C., Calus, A., Degroot, J. M. and Verhe, R., 2006, Influence of fertilization on acrylamide formation during frying of potatoes harvested in 2003. *Journal of Agricultural and Food Chemistry*, 54, 404-408.
- Eppendorfer, W. H. and Bille, S. W., 1996, Free and total amino acid composition of edible parts of beans, kale, spinach, cauliflower and potatoes as influenced by nitrogen

- fertilisation and phosphorus and potassium deficiency. *Journal of the Science of Food and Agriculture*, 71, 449-458.
- Food Standards Agency, 2006, Draft pesticide residue minimisation guides for five crops (London: Food Standards Agency).
- Garthwaite, A. J., von Bothmer, R. and Colmer, T. D., 2005, Salt tolerance in wild *Hordeum* species is associated with restricted entry of Na⁺ and Cl⁻ into the shoots. *Journal of Experimental Botany*, 56, 2365-2378.
- Good, A.G. and Zaplachinski, S. T., 1994, The effects of drought stress on free amino acid accumulation and protein synthesis in *Brassica napus*. *Physiologia Plantarum*, 90, 9-14.
- Halford, N.G., 2006, Regulation of carbon and amino acid metabolism: roles of sucrose nonfermenting-1-related protein kinase-1 and general control nonderepressible-2-related protein kinase. *Advances in Botanical Research Incorporating Advances in Plant Pathology*, 43, 93-142.
- Halford, N.G. and Paul, M.J., 2003, Carbon metabolite sensing and signalling. *Plant Biotechnology Journal*, 1, 381-398.
- Jung, M. Y., Choi, D. S. and Ju, J. W., 2003, A novel technique for limitation of acrylamide formation in fried and baked corn chips and in French fries. *Journal of Food Science*, 68, 1287-1290.
- Lea, P. J. and Mifflin, B. J., 2003, Glutamate synthase and the synthesis of glutamate in plants. *Plant Physiology and Biochemistry*, 41, 555-564.
- Lea, P. J., Sodek, L., Parry, M. A., Shewry, P. R. and Halford, N. G., 2007, Asparagine in plants. *Annals of Applied Biology*, 150, 1-26.
- Matsuura-Endo, C., Ohara-Takada, A., Chuda, Y., Ono, H., Yada, H., Yoshida, M., Kobayashi, A., Tsuda, S., Takigawa, S., Noda, T., Yamauchi, H. and Mori, M., 2006,

- Effects of storage temperature on the contents of sugars and free amino acids in tubers from different potato cultivars and acrylamide in chips. *Bioscience, Biotechnology and Biochemistry*, 70, 1173-1180
- McKibbin, R. S., Muttucumaru, N., Paul, M. J., Powers, S. J., Burrell, M. M., Coates, S., Purcell, P. C., Tiessen, A., Geigenberger, P. and Halford, N. G., 2006, Production of high starch, low glucose potatoes through over-expression of the metabolic regulator, SnRK1. *Plant Biotechnology Journal*, 4, 409-418.
- Mottram, D. S., Wedzicha, B. L. and Dodson, A. T., 2002, Acrylamide is formed in the Maillard reaction. *Nature*, 419, 448-449.
- Morrell, S. and Ap Rees, T., 1986, Sugar metabolism in developing tubers of *Solanum tuberosum*. *Phytochemistry*, 25, 1579-1585.
- Mucci, L. A., Dickman, P. W., Steineck, G., Adami, H. O. and Augustsson, K., 2003, Dietary acrylamide and cancer of the large bowel, kidney, and bladder: absence of an association in a population-based study in Sweden. *British Journal of Cancer*, 88, 84-89.
- Muttucumaru, N., Halford, N. G., Elmore, J. S., Dodson, A. T., Parry, M. A. J., Shewry, P. R. and Mottram, D.S., 2006, The formation of high levels of acrylamide during the processing of flour derived from sulfate-deprived wheat. *Journal of Agricultural and Food Chemistry*, 54, 8951-8955.
- Nikiforova, V. J., Bielecka, M., Gakiere, B., Krueger, S., Rinder, J., Kempa, S., Morcuende, R., Scheible, W.R., Hesse, H. and Hoefgen, R., 2006, Effect of sulphur availability on the integrity of amino acid biosynthesis in plants. *Amino Acids*, 30, 173-183.
- Olsson, K., Svensson, R. and Roslund, C. A., 2004, Tuber components affecting acrylamide formation and colour in fried potato: variation by variety, year, storage temperature and storage time. *Journal of the Science of Food and Agriculture*, 84, 447-458.

- Pelucchi, C., Franceschi, S., Levi, F., Trichopoulos, D., Bosetti, C., Negri, E. and La Vecchia, C., 2003, Fried potatoes and human cancer. *International Journal of Cancer*, 105, 558-560.
- Perez-Garcia, A., Pereira, S., Pissarra, J., Gutierrez, A. G., Cazorla, F. M., Salema, R., de Vicente, A. and Canovas, F.M., 1998, Cytosolic localization in tomato mesophyll cells of a novel glutamine synthetase induced in response to bacterial infection or phosphinothricin treatment. *Planta*, 206, 426-434.
- Possingham, J.V., 1956, The effect of mineral nutrition on the content of free amino acids and amides in tomato plants. I. A comparison of the effects of deficiencies of copper, zinc, manganese, iron and molybdenum. *Australian Journal of Biological Sciences*, 9, 539-551.
- Rabe, E. and Lovatt, C. J., 1986, Increased arginine biosynthesis during phosphorus deficiency. *Plant Physiology*, 81, 774-779.
- Rommens, C. M., Ye, J., Richael, C. and Swords, K., 2006, Improving potato storage and processing characteristics through all-native DNA transformation. *Journal of Agricultural and Food Chemistry*, 54, 9882-9887.
- Rufty, T. W., MacKown, C. T. and Israel, D. W., 1990, Phosphorus stress effects on the assimilation of nitrate. *Plant Physiology*, 94, 328-333.
- Rydberg, P., Eriksson, S., Tareke, E., Karlsson, P., Ehrenberg, L. and Törnqvist, M., 2003, Investigations of factors that influence the acrylamide content of heated foodstuffs. *Journal of Agriculture and Food Chemistry*, 51, 7012-7018.
- Scarpari, L. M., Meinhardt, L. W., Mazzafera, P., Pomella, A. M. V., Schiavinato, M. A., Cascardo, J. C. M. and Pereira, G. A. G., 2005, Biochemical changes during the development of witches' broom: the most important disease of cocoa in Brazil caused by *Crinipelis perniciosa*. *Journal of Experimental Botany*, 56, 865-877.

- Shewry, P. R., Franklin, J., Parmar, S., Smith, S. J. and Mifflin, B. J., 1983, The effects of sulphur starvation on the amino acid and protein compositions of barley grain. *Journal of Cereal Science*, 1, 21-31.
- Shewry, P.R., Tatham, A.S. and Halford, N.G., 2001, Nutritional control of storage protein synthesis in developing grain of wheat and barley. *Plant Growth Regulation*, 34, 105-111.
- Smirnoff, N. and Stewart, G. R., 1987, Nitrogen assimilation and zinc toxicity to zinc-tolerant and non-tolerant clones of *Deschampsia cespitosa* (L.) Beauv. *New Phytologist*, 107, 671-680.
- Stadler, R. H., Blank, I., Varga, N., Robert, F., Hau, J., Guy, P. A., Robert, M.-C. and Riediker, S., 2002, Acrylamide from Maillard reaction products. *Nature*, 419, 449-450.
- Stewart, G. R. and Larher, F., 1980, Accumulation of amino acids and related compounds in relation to environmental stress. *The Biochemistry of Plants, Volume 5*, edited by B. J. Mifflin (London: Academic Press), pp. 609-635.
- Surdyk, N., Rosén, J., Andersson, R. and Åman, P., 2004, Effects of asparagine, fructose, and baking conditions on acrylamide content in yeast-leavened wheat bread. *Journal of Agriculture and Food Chemistry*, 52, 2047-2051.
- Taeymans, D., Wood, J., Ashby, P., Blank, I., Studer, A., Stadler, R. H., Gonde, P., Van Eijck, P., Lalljie, S., Lingnert, H., Lindblom, M., Matissek, R., Muller, D., Tallmadge, D., O'Brien, J., Thompson, S., Silvani, D., and Whitmore, T., 2004, A review of acrylamide: An industry perspective on research, analysis, formation and control. *Critical Reviews in Food Science and Nutrition*, 44, 323-347.
- Taubert, D., Harlfinger, S., Henkes, L., Berkels, R. and Schömig, E., 2004, Influence of processing parameters on acrylamide formation during frying of potatoes. *Journal of Agriculture and Food Chemistry*, 52, 2735-2739.

- Trethewey, R. N., Geigenberger, P., Riedel, K., Hajirezaei, M-R., Sonnewald, U., Stitt, M., Riesmeier, J. W. and Willmitzer, L., 1998, Combined expression of glucokinase and invertase in potato tubers leads to a dramatic reduction in starch accumulation and a stimulation of glycolysis. *Plant Journal*, 15, 109-118.
- Wedzicha, B. L., Mottram, D. S., Elmore, J. S., Koutsidis, G. and Dodson, A.T., 2005, Kinetic models as a route to control acrylamide formation in food. *Advances in Experimental Medicine and Biology*, 561, 235-253.
- White, M. C., Baker, F. D., Chaney, R. L. and Decker, A. M., 1981, Metal complexation in xylem fluid II. Theoretical equilibrium model and computational computer program. *Plant Physiology*, 67, 301-310
- Winkler, U. and Schön, W. J., 1980, Amino acid composition of the kernel proteins in barley resulting from nitrogen fertilization at different stages of development. *Journal of Agronomy and Crop Science*, 149, 503-512.
- Zhao, F. J., Hawkesford, M. J. and McGrath, S. P., 1999, Sulphur assimilation and effects on yield and quality of wheat. *Journal of Cereal Science*, 30, 1-17.
- Zhao, F. J., McGrath, S. P., Blake-Kalff, M. M. A. and Link, A. and Tucker, M.R., 2002, Crop responses to sulphur fertilisation in Europe. *International Fertiliser Society Proceeding No 504* (York: The International Fertiliser Society).
- Zhang, Y., Dickinson, J. R., Paul, M. J. and Halford, N. G., 2003, Molecular cloning of an arabidopsis homologue of GCN2, a protein kinase involved in co-ordinated response to amino acid starvation. *Planta*, 217, 668-675.
- Zyzak, D. V., Sanders, R. A., Stojanovic, M., Tallmadge, D. H., Eberhart, B. L., Ewald, D. K., Gruber, D. C., Morsch, T. R., Strothers, M. A., Rizzi, G. P. and Villagran, M. D., 2003, Acrylamide formation mechanism in heated foods. *Journal of Agriculture and Food Chemistry*, 51, 4782-4787.

Figures.

Figure 1. A bag of superphosphate (a mixture of dihydrogen phosphate and hydrated calcium sulphate) from the mid-20th century depicting an image of Sir John Bennett Lawes of Rothamsted, who began manufacturing superphosphate in 1841. The manufacturing process involved dissolving first bones and later mineral phosphates in sulphuric acid, and superphosphate was therefore a good source of sulphur. The move to more modern fertilizers based on nitric acid has contributed to sulphur deficiency.



Figure 2. Analysis of wholemeal flour from three varieties of winter wheat grown under glass with or without sulphur (S) feeding (data of Muttucumaru *et al.*, 2006).

Top: Asparagine concentration. Middle: Acrylamide formed during processing. Bottom: Correlation between asparagine content and acrylamide formation.

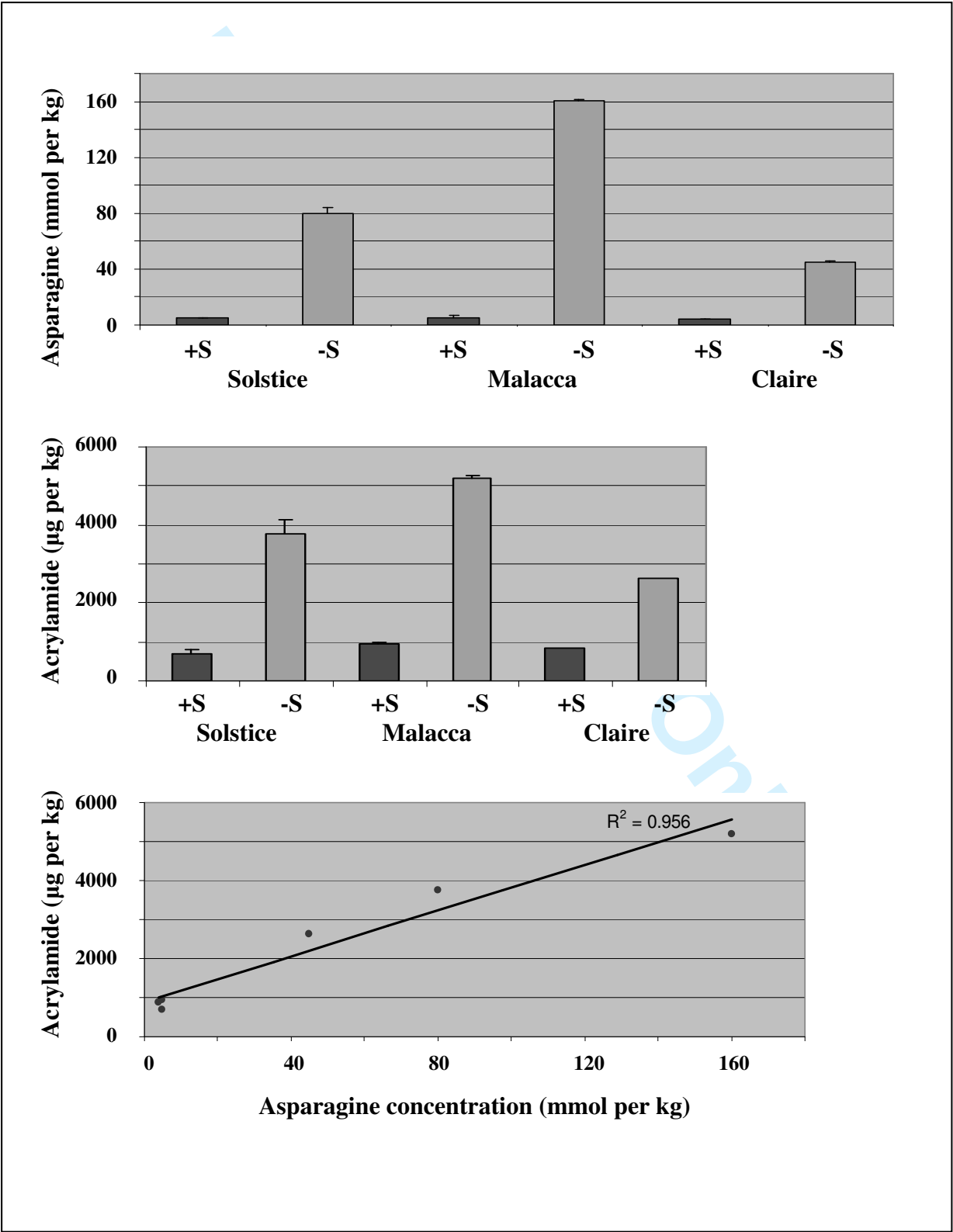
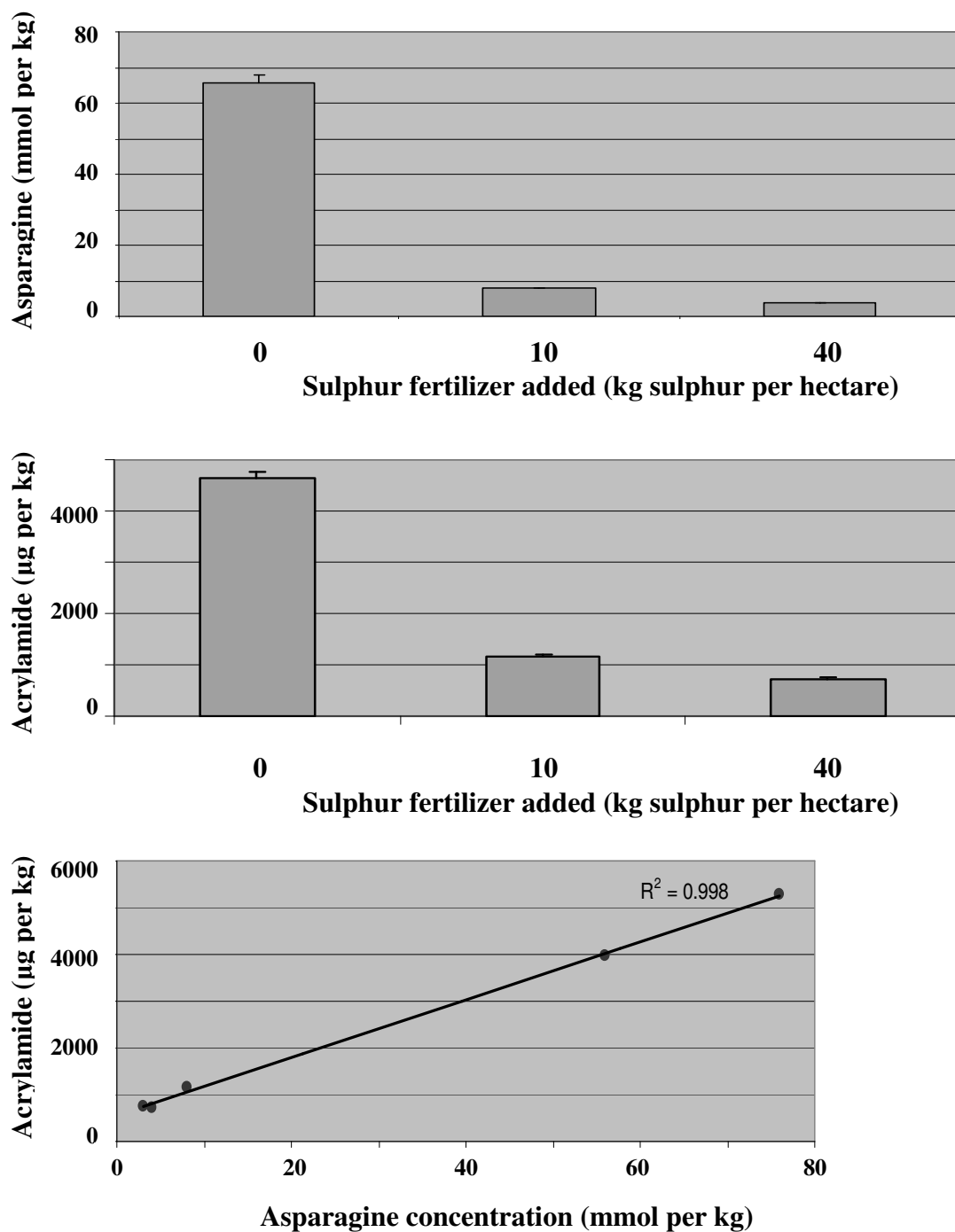


Figure 3. Analysis of wholemeal flour from wheat (cv Hereward) grown in the field with three levels of sulphur (S) feeding, as indicated (data of Muttucumaru *et al.*, 2006).

Top: Asparagine concentration. Middle: Acrylamide formed during processing. Bottom:

Correlation between asparagine content and acrylamide formation.



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Figure 4. a) Wheat plants at harvest after cultivation with sulphur (provided during watering in the form of 1 mM MgSO₄) (left panel) or without sulphur (right panel). b) Potato plants (top left), roots (top right) and tubers (bottom left) after growth with sulphur (S) (applied as in (a)) or without sulphur, as indicated.

a)



b)

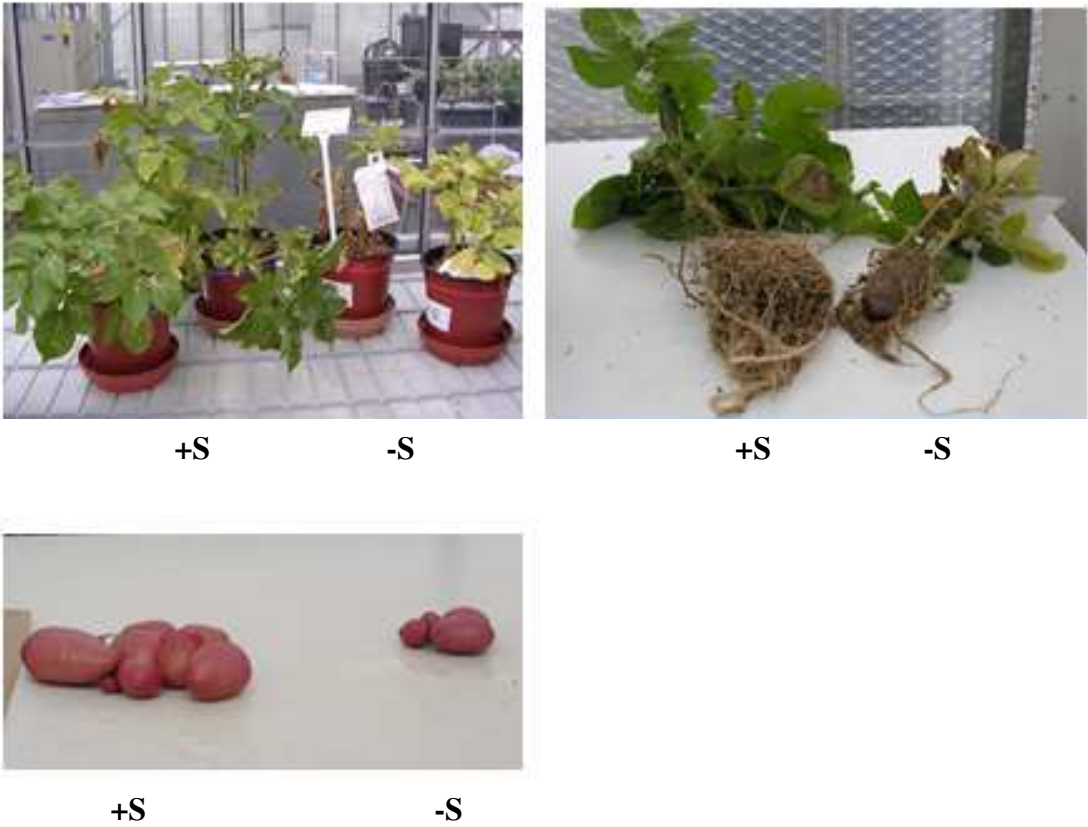


Figure 5. Simple schematic showing the participation of amino acids in the Maillard reaction

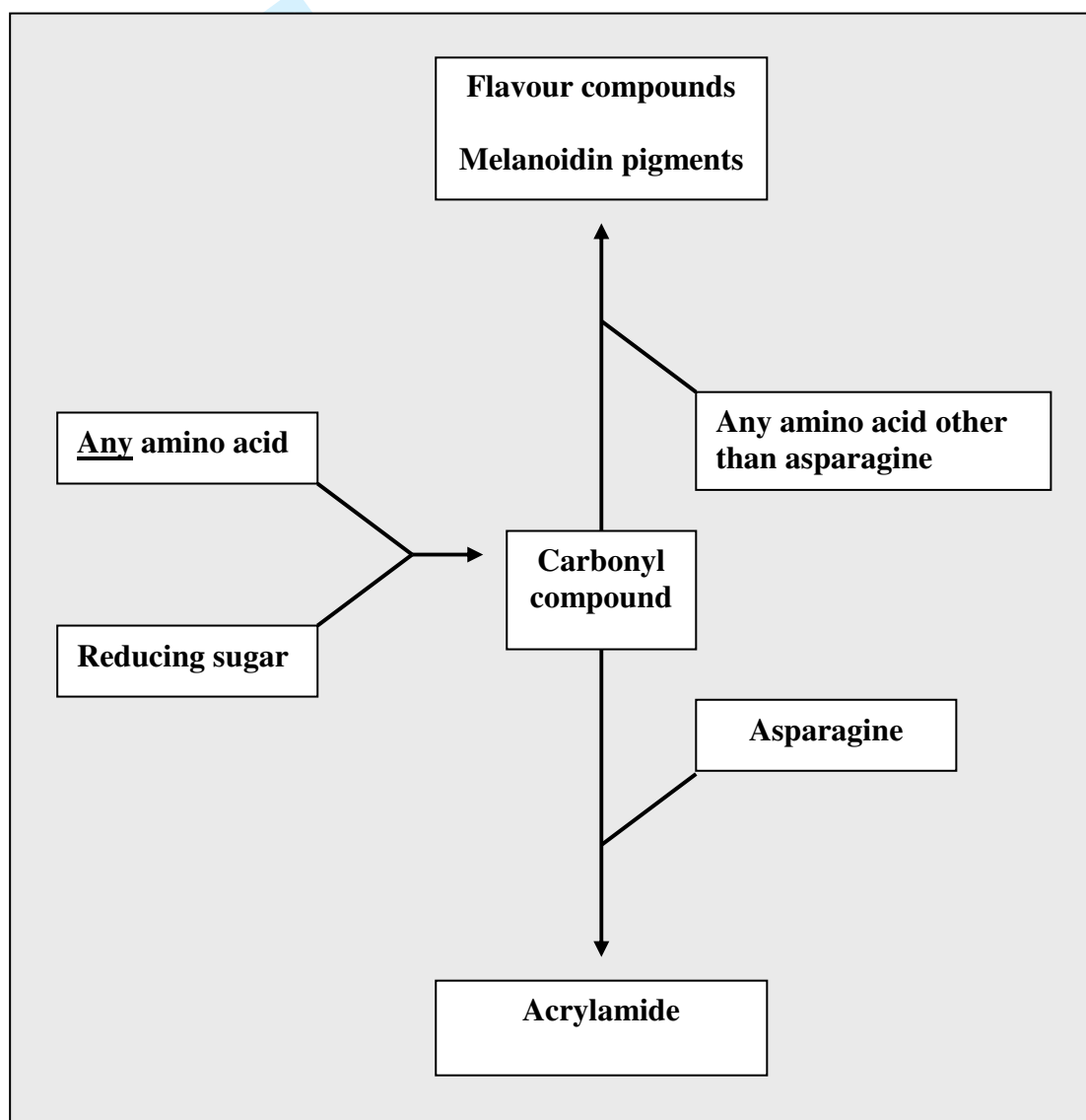


Figure 6. Asparagine synthesis and break-down

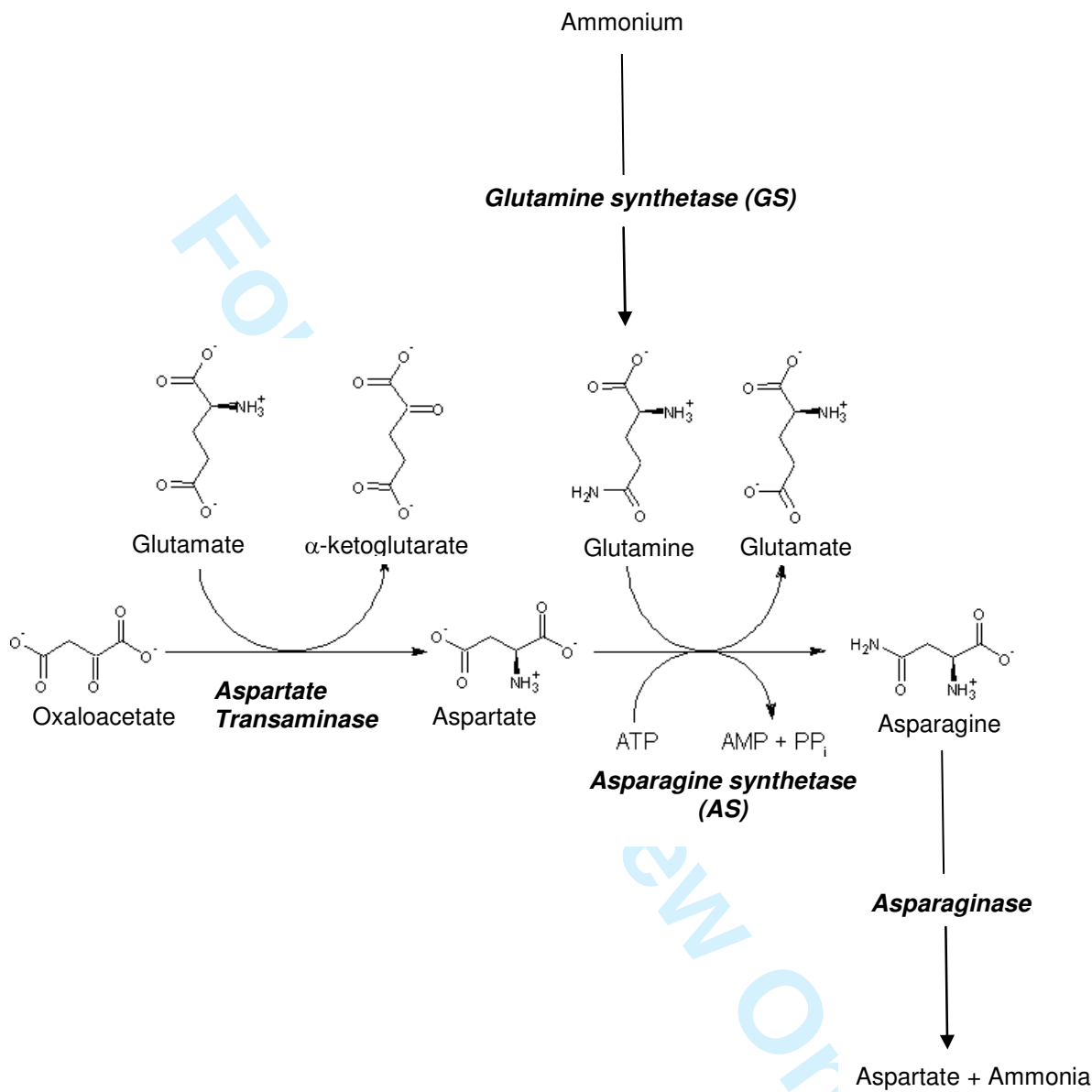


Figure 7. Schematic showing the inter-relationship of sugars in potato tubers during development and the key enzymes involved. SnRK1 (sucrose nonfermenting-1-related protein kinase 1) is a metabolic regulator that channels carbon through the storage as opposed to glycolytic pathway (McKibbin *et al.* 2006).

