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## Acrylamide in cereal and cereal products: a review on progress in mitigation.

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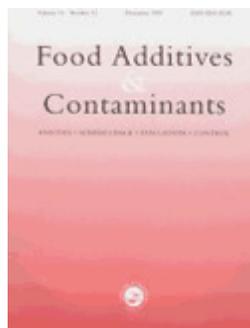
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**Acrylamide in cereal and cereal products: a review on progress in mitigation.**

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Manuscripts

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2 1 **Acrylamide in cereal and cereal products: a review on progress in**  
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4 2 **mitigation.**  
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34 18 Running title: acrylamide mitigation in cereal products  
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4 2 **Abstract**  
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8 4 In March 2006 a joint workshop was organized by the European Commission and the  
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10 5 Confederation of EU food and drink industries (CIAA) to discuss the key knowledge and  
11  
12 6 achievements in the mitigation of acrylamide. This paper focuses on the progress made with  
13  
14 7 cereal products. At present the mitigation options available are applicable to a limited  
15  
16 8 number of cereal products and are product specific. The following are the most promising:  
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18 9

- 19 10 • Adjustment of time and temperature during baking.
- 20 11 • Extend fermentation times where feasible.
- 21 12 • Substitution of ammonium bicarbonate with alternatives where this is feasible.
- 22 13 • Avoid or minimise use of reducing sugars where possible.
- 23 14 • Maintenance of uniform control of the colour and avoidance of very high baking  
24 15 temperature where possible.

25 16 The most promising near term technical solution is the use of asparaginase. This enzyme  
26 17 has potential to achieve 60-90% reductions for some products made from dough or batters  
27 18 which can be held for a time. In the longer term the optimisation of agronomy and plant  
28 19 breeding for wheat has potential to reduce acrylamide for all foods on any scale whether  
29 20 domestic or industrial. Importantly, nutritional and toxicological issues other than acrylamide  
30 21 must also be considered so as to ensure that steps taken to reduce acrylamide do not have  
31 22 other adverse effects on the diet.  
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42 23 **Keywords** :- Acrylamide, asparagine, asparaginase, cereals, cereal products, mitigation,  
43 24 processing, recipe, yeast.  
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## 1 Introduction

2 In a status report of December 2004, the Confederation of the food and drink industries of  
3 the EU (CIAA) reported the progress made until that date in lowering acrylamide levels in the  
4 major food categories of concern (CIAA 2004). In March 2006 a joint workshop was  
5 organized by the European Commission and the CIAA for government, industry and  
6 academia to discuss the key knowledge and achievements to date, results of the current  
7 studies and projects, opportunities, gaps and constraints. One aim of the workshop was to  
8 update the toolbox approach on acrylamide (CIAA 2005) and this was published in October  
9 2006 (CIAA 2006a).

10  
11 Following the workshop, a number of detailed actions were agreed at the latest Expert  
12 Committee on Environmental and Industrial Contaminants meeting of the EU Commission so  
13 as to ensure that voluntary measures such as the toolbox approach are effectively applied  
14 by food operators and their effects monitored. These actions include the development of a  
15 European monitoring programme, contribution to the development and future distribution of  
16 sector specific brochures giving guidance to small and medium sized food operators on how  
17 to take steps to minimise acrylamide formation, and the development of a European  
18 framework on consumer advice for home cooking. These actions will be carried out in close  
19 co-operation with the relevant stakeholder organisations (SANCO 2006). Since 2003 both  
20 the European Commission and the World Health Organization posted calls for data and  
21 placed their spreadsheets for the submission of data on the web. The goal of the databases  
22 is to collect data for a reliable estimation of the exposure of consumers to acrylamide via the  
23 food chain (Lineback et al., 2005)

24  
25 This paper focuses on issues, achievements and results of current studies and projects on  
26 cereal and cereal products since the publication of the CIAA toolbox in 2005. Furthermore,  
27 aspects of future issues and gaps in knowledge are discussed.

1  
2 **1 Progress to date**  
3

4 2  
5 3 Cereal foods encompass a vast range of different products with many ingredients,  
6 4 processes, recipes and scales of operation. Research has confirmed that there is no simple  
7 5 route to reduce acrylamide. To date a limited number of successes, for example through  
8 6 work on raising agents, have been achieved. Asparaginase also looks very promising for the  
9 7 near future and longer-term work on generating cereal varieties naturally low in asparagine  
10 8 may prove successful  
11 9

12 10 Within the CIAA toolbox, four key compartments encompassing 13 different parameters  
13 11 have been listed with the potential for reduction of acrylamide:

- 14 12 - Agronomical
- 15 13 - Recipe
- 16 14 - Processing
- 17 15 - Final preparation

18 16 Stadler and Scholz (2004) and Stadler (2005) summarized the most important findings with  
19 17 regard to formation and possible mitigation of acrylamide in cereal products and bakery  
20 18 products. Raw materials, ingredients and processing conditions were taken into account.  
21 19 The text below focuses on issues since that date.

22 20  
23 21 *Kinetic / predictive modelling tools*  
24 22

25 23 It is now widely accepted that acrylamide is primarily formed in cereal products from  
26 24 asparagine reacting with an intermediate, which is typically a carbonyl source formed from a  
27 25 reaction between amino acids and reducing sugars. The simplest possible reaction scheme  
28 26 for the formation of acrylamide is summarised in figure 1.

29 27 **[insert Figure 1 about here]**

30 28 The value of these simplified reaction schemes or kinetic models lies in their predictive  
31 29 applications for “what-if” experiments to determine conditions that can lead to reduced  
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2 1 acrylamide. Sadd and Hamlet (2005) and Wedzicha et al. (2005) validated traditional kinetic  
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4 2 models for the formation and loss of acrylamide in wheat and rye products and  
5  
6 3 demonstrated that levels of acrylamide in cereal products are limited by amino acids running  
7  
8 4 out as well as direct decay or loss of the acrylamide produced. Corradini and Peleg 2006  
9  
10 5 used mathematical simulations based on Fermian decay to demonstrate how temperature  
11  
12 6 can be controlled to minimise acrylamide without losing the desirable effects of a heat  
13  
14 7 process. Perhaps the most interesting finding from these model studies, however, was that  
15  
16 8 the levels of Maillard products (browning) do not necessarily align with acrylamide levels  
17  
18 9 (Sadd and Hamlet 2005; Hamlet et al. 2005b); low moisture content is a more important  
19  
20 10 promoter of acrylamide than temperature, so crust moisture is a key factor controlling  
21  
22 11 acrylamide levels, and this accounts for much of the variation seen between different types  
23  
24 12 of cereal products.

#### 25 14 *Agronomical parameters*

26  
27 15 The determinant factor for acrylamide in cereal products is free asparagine. The free  
28  
29 16 asparagine content varies widely. There is not enough information available to determine a  
30  
31 17 clear trend: wheat has been reported at 69 to 443 mg kg<sup>-1</sup>, while in rye the concentration  
32  
33 18 ranged from 319-791 mg kg<sup>-1</sup> (Amrein et al., 2004; Springer et al. 2003; Taeymans et al.,  
34  
35 19 2004; Hamlet et al. 2005). . Investigations of Claus et al. (2006) (unpublished data) revealed  
36  
37 20 a significant impact of cultivar and fertilisation on acrylamide levels in bakery products due to  
38  
39 21 variations in the asparagine and crude protein contents of the raw material. A recent  
40  
41 22 publication (Muttucumaru et al. 2006) suggests that sulphate deficiency in soils causes  
42  
43 23 wheat to accumulate asparagine. The effects of sulphate were greater than varietal  
44  
45 24 differences.

46 25 Breakfast cereal manufacturers using wheat sourced from the neighbourhood of their  
47  
48 26 factories have observed significant changes in acrylamide content from season to season as  
49  
50 27 shown in table 1 and the CIAA status report 2004 (CIAA 2004).

51 28 **[insert table 1 about here]**

1  
2 1 The implication is that the asparagine content of wheat is significantly influenced by  
3  
4 2 seasonal factors.

5  
6 3 Earlier studies showed that the ash content of flour influenced acrylamide content in  
7  
8 4 bakery products. Flours high in ash have significantly elevated amino acid contents, resulting  
9  
10 5 in higher acrylamide contents. Furthermore, dusting of the breads with flour prior to baking  
11  
12 6 elevates acrylamide contents significantly (Claus et al. 2006).  
13  
14 7

15 8 Aspects of recipe and process are discussed for biscuits, bread and breakfast  
16  
17 9 cereals, as progress in mitigation due to changes in these key parameters may differ for  
18  
19 10 each product group.  
20  
21 11

#### 22 12 *Recipe and process parameters*

- 23 13 • Biscuits

24  
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26 14  
27 15 Figure 2 shows the effect of recipe changes on the acrylamide content of wheat crackers  
28  
29 16 (Vass et al. 2004).  
30

31 17 **[insert figure 2 about here]**

32  
33 18 Vass et al. (2004) concluded that the application of an asparaginase during dough  
34  
35 19 preparation can reduce the acrylamide content of cracker products by at least 70%. The  
36  
37 20 baking agent ammonium hydrogencarbonate ( $\text{NH}_4\text{HCO}_3$ ) promotes acrylamide formation  
38  
39 21 and its replacement is another way to limit acrylamide. Alternatives, which have been tried  
40  
41 22 with varying levels of success, include sodium bicarbonate +/- acidulants in combination with  
42  
43 23 other changes, disodium diphosphate, sodium bicarbonate and organic acids or potassium  
44  
45 24 bicarbonate with potassium bitartrate. However these alternatives are generally slower to  
46  
47 25 generate gas and often require acid addition to speed carbonation and can leave a residue  
48  
49 26 which may create adverse flavours, texture and colour. Minimizing reducing sugars in the  
50  
51 27 ingredients or the use of a sucrose solution instead of inverted sugar syrup can also help to  
52  
53 28 produce crackers with lower acrylamide content. Two earlier papers reported the effect of

Formatted

1  
2 1 carbonate or bicarbonate salts on acrylamide formation in wheat based model systems  
3  
4 2 (Amrein et al. 2004; Biedermann et al. 2003) and both found that ammonium bicarbonate  
5  
6 3 greatly enhanced the amount of acrylamide in recipes containing added reducing sugar. It  
7  
8 4 was noted that both ammonium and added reducing sugar was required to enhance the  
9  
10 5 acrylamide formation mechanism. Levine and Smith (2005) reported that ammonium  
11  
12 6 bicarbonate in a recipe, which did not contain added reducing sugar failed to give an  
13  
14 7 increase in acrylamide relative to the basic wheat/water recipe. This is important since the  
15  
16 8 added sugar used in the majority of biscuit recipes is sucrose, a non-reducing sugar.  
17  
18 9 Cysteine, sodium bisulfite, and ascorbate also enhanced elimination. Asparagine, but not  
19  
20 10 reducing sugar, caused a large increase in acrylamide formation.

21  
22 11 Amrein et al. (2004) reported that ammonium bicarbonate strongly enhanced  
23  
24 12 acrylamide formation in gingerbread and that replacement with sodium hydrogencarbonate  
25  
26 13 as baking agent reduced the acrylamide concentration by >60%. Free asparagine was a  
27  
28 14 limiting factor for acrylamide formation, but the acrylamide content could also be lowered by  
29  
30 15 replacing reducing sugars with sucrose or by adding organic acids. Acrylamide concentration  
31  
32 16 and browning intensity both increased with baking time and correlated with each other.

33  
34 17 Results from monitoring activities of the Food and Consumer Product Safety  
35  
36 18 Authority (VWA) in the Netherlands showed a decrease ( $p < 0.05$ ) in acrylamide levels of  
37  
38 19 Dutch spiced cake available on the Dutch market in 2006 (table 2). According to the  
39  
40 20 information on the product label, ammonium bicarbonate is not used anymore in products  
41  
42 21 analysed in 2006, while product labels of the same products in 2003, which were high in  
43  
44 22 acrylamide, showed the use of ammonium bicarbonate. Dutch spiced cake accounts for  
45  
46 23 16% of the total acrylamide exposure from foods for the whole population in the Netherlands  
47  
48 24 (Konings et al. 2003).

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**[insert table 2 about here]**

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In the UK an on-going study (Hamlet et al. 2005) is looking at the relationship  
between acrylamide in retail products and declared raising agents, and researching ways of  
counteracting the negative effects of added ammonium.

1  
2 1 Graf et al. (2006) showed that replacement of ammonium bicarbonate reduced  
3  
4 2 acrylamide by 70% in semi-finished biscuits at an industrial scale. The use of sucrose  
5  
6 3 instead of inverted sugar syrup had a similar effect. Addition of extra tartaric acid reduced  
7  
8 4 acrylamide by about one-third.

9  
10 5 The addition of ingredients inhibiting acrylamide formation by reaction with precursors  
11  
12 6 of acrylamide may be more suitable, eg. flavonoids in apples, tea leaves and onions might  
13  
14 7 react with asparagine (Claus et al. 2006).

15 8 A wide range of different processing techniques are used in the manufacture of  
16  
17 9 biscuits in order to arrive at the different shapes, textures and flavours typical of such  
18  
19 10 disparate products as shortbread, sandwich creams, crackers, soft cookies, digestives,  
20  
21 11 crackers, gingerbreads crispbreads etc. The influence of the different mixing and forming  
22  
23 12 operations on acrylamide levels in the finished product is far from understood. Some  
24  
25 13 multifactorial experiments carried out by Danone looking at the role of mixing time, pH and  
26  
27 14 fructose content at the dough mixing stage of a model short sweet biscuit recipe have shown  
28  
29 15 that these factors interact strongly to influence final acrylamide levels, but without a clear  
30  
31 16 explanation for the magnitude or the direction of the effects. The work does highlight  
32  
33 17 however the impact which the condition of the dough during processing can have on  
34  
35 18 acrylamide production and perhaps suggests that the state of ingredients entering the mixer  
36  
37 19 – syrup, powder, solution – and the impact of physical factors such as work and temperature  
38  
39 20 on the dough are areas needing further study. That they do influence acrylamide formation is  
40  
41 21 probably one of the reasons why measured acrylamide in different batches of the same  
42  
43 22 finished product are found to vary so widely.

44 23 , Although changes in the oven baking profile resulted in significant reduction of  
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46 24 acrylamide in non-fermented crispbread, less positive results have been achieved with other  
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48 25 biscuit products. Most manufacturers who have optimized their oven process so as to  
49  
50 26 increase heat flux in zone 1 and/or decrease it in later zones to balance the overall heat flux,  
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52 27 have found that reductions in acrylamide are marginal (10-20%) and that they must also  
53  
54 28 accept an increase in product moisture and/or a lighter colour in order to achieve this

1  
2 1 reduction. It also must often be accompanied by adjustments to raising agents and dosing  
3  
4 2 points, dough forming modifications and extended baking times. Given the substantial  
5  
6 3 acrylamide variability between and within batches of biscuits, often +/- 20%, these mitigation  
7  
8 4 measures based on changes to oven baking profile look weak.  
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10 5

11 6 • Crispbread  
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15 8 Fermented crispbread generally contains substantially less acrylamide than non-  
16  
17 9 fermented crispbread, but each has it's own distinctive characteristics (CIAA, 2006b).

18  
19 10 Changes to the oven baking profile have been suggested as one possible route towards  
20  
21 11 reduction of acrylamide (CIAA Toolbox), and a recent survey carried out by the EU  
22  
23 12 Association of Chocolate, Biscuit and Confectionery Industries (CAOBISCO) assessed the  
24  
25 13 extent to which manufacturers have found success with this, as with other, possible  
26  
27 14 mitigation measures. In the case of non-fermented crispbread changes to the oven profile  
28  
29 15 have led to significant (25 – 75%) reduction in acrylamide.

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31 16 Mustafa et al. (2005) described that added asparagine had a significant effect on the  
32  
33 17 formation of acrylamide in rye crispbread, although surprisingly the addition of fructose did  
34  
35 18 not have an effect on the formation of acrylamide. Added oat-bran concentrate was added  
36  
37 19 for its water-binding capacity, and the enzyme lichenase was used to selectively degrade  $\beta$ -  
38  
39 20 glucan into fragments that were expected to influence viscosity and water-binding capacity,  
40  
41 21 but these did not influence acrylamide content.  
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44 23  
45 24 • Bread  
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49 26 In the UK most commercial bread is produced by a rapid mechanical dough  
50  
51 27 development procedure, i.e. the Chorleywood baking process (Chamberlain et al. 1962) and  
52  
53 28 the levels of acrylamide formed in these breads are typically very low (Food Standards  
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1  
2 1 Agency 2005). Hamlet et al. (2003; 2005b; 2006) investigated the factors contributing to the  
3  
4 2 low levels of acrylamide generated in UK commercial and domestic breads. In addition to a  
5  
6 3 dilution of acrylamide (formed exclusively in the crust) by the acrylamide free crumb, it was  
7  
8 4 shown that the Statutory addition of calcium (carbonate) to flour (see figure 7), yeast  
9  
10 5 fermentation (see figure 8) and oven profiling were the main controlling parameters.

11 6 Extensive fermentation with yeast may be one possible way to reduce acrylamide  
12  
13 7 content in bread by reducing free asparagine (Fredriksson et al. 2004; Hamlet et al. 2005a,  
14  
15 8 b), an important precursor for acrylamide in cereal products. The content of free asparagine  
16  
17 9 was determined by Fredriksson (2004) in 11 milling fractions from wheat and rye. Whole  
18  
19 10 grain wheat flour contained  $0.5 \text{ g kg}^{-1}$  and whole grain rye flour  $1.1 \text{ g kg}^{-1}$ . The lowest content  
20  
21 11 was found in sifted wheat flour ( $0.2 \text{ g kg}^{-1}$ ). Wheat germ had the highest content ( $4.9 \text{ g kg}^{-1}$ ).  
22  
23 12 Fermentation (baker's yeast or baker's yeast and sourdough) of doughs made with the  
24  
25 13 different milling fractions was performed to investigate whether the content of free  
26  
27 14 asparagine was reduced by this process. In general, most of the asparagine was utilized  
28  
29 15 after 2 hr of fermentation with yeast. Sourdough fermentation, on the other hand, did not  
30  
31 16 reduce the content of free asparagine as efficiently but had a strong negative impact on  
32  
33 17 asparagine utilization by yeast. This indicated that this type of fermentation may result in  
34  
35 18 breads with higher acrylamide content than in breads fermented with yeast only. The effect  
36  
37 19 of fermentation time on acrylamide formation in yeast-leavened bread was studied in a  
38  
39 20 model system. Compared with short fermentation time, longer fermentation reduced  
40  
41 21 acrylamide content in bread made with whole grain wheat 87%. For breads made with rye  
42  
43 22 bran, the corresponding reduction was 77%.

44 23 In laboratory and small scale trials several groups have reported that added amino  
45  
46 24 acids can reduce acrylamide (Amrein et al 2004; Bråthen et al. 2005). Cysteine competes  
47  
48 25 with asparagine for reducing sugars in the Maillard reaction; and the thiol group may react  
49  
50 26 with the double bond of acrylamide. On the other hand its application in dough is limited  
51  
52 27 because cysteine also reacts with gluten proteins, which has a significant impact on the  
53  
54 28 rheological properties of the wheat gluten network and hence the quality of the product.

1  
2 1 Bråthen et al. (2005) described that the addition of glycine during dough making significantly  
3  
4 2 reduced acrylamide in both flat breads and bread crusts. In bread crusts the reduction of  
5  
6 3 acrylamide ranged from 50 to >90%, depending on the baking condition. In flat bread the  
7  
8 4 reduction varied between 60 and >95%. However these reductions require relatively high  
9  
10 5 dosages of amino acids, e.g. 80-400 mmol kg<sup>-1</sup> (Hamlet 2005b), and unpleasant odours can  
11  
12 6 occur at these levels of addition (Claus et al. 2006).

13 7 The pH value of the dough is another factor affecting acrylamide formation. Lowering  
14  
15 8 the pH values in dough can decrease acrylamide (Claus et al. 2006; Hamlet et al 2005b).  
16  
17 9 This can be accomplished by adding consumable acids such as citric acid or by lactic  
18  
19 10 fermentation, as applied during sourdough preparation. It is well known that pH is an  
20  
21 11 important determinant of the rate and formation of key Maillard reaction intermediates, e.g.  
22  
23 12 Schiff base formation and the acidic medium can also lead to deamination of asparagine.  
24  
25 13 The practical pH range required to produce viable bread dough is however very limited and  
26  
27 14 hence this application may be more suitable as a surface treatment.

28 15 Surdyk et al. (2004) studied the effects of asparagine, fructose and baking conditions  
29  
30 16 on acrylamide content in yeast-leavened wheat bread. More than 99% of the acrylamide was  
31  
32 17 found in the crust. Added asparagine dramatically increased the content of acrylamide in  
33  
34 18 crusts dry matter, while added fructose did not influence the content. The effects of  
35  
36 19 temperature and time of baking were studied in another experiment. Mainly temperature  
37  
38 20 (above 200°C) but also time increased the acrylamide content in crust dry matter, and a  
39  
40 21 significant interaction was seen between these two factors. When baked at different  
41  
42 22 conditions with the same ingredients, a highly significant relationship between colour and  
43  
44 23 acrylamide content in crust was found. Added asparagine, however, did not increase colour.

45 24 Time and temperature parameters are strongly affecting acrylamide formation. In  
46  
47 25 order to obtain products that are low in acrylamide, while still being acceptable to the  
48  
49 26 consumer, reduced temperatures and prolonged baking times should be given priority (Claus  
50  
51 27 et al. 2006). However, acrylamide is subject to partial degradation when the surface

1  
2 1 temperature exceeds 180°C, probably due to polymerisation (Claus et al. 2006) and  
3  
4 2 volatilisation.

5  
6 3 Acrylamide formation not only depends on temperature as such, but also on heat transfer to  
7  
8 4 the product. Tunnel or travelling ovens are used for large scale commercial bread production  
9  
10 5 (e.g. in the UK) and these can use both radiant and convective heat transfer. For smaller  
11  
12 6 scale bread production, two types of oven are usually used. In the traditional deck oven heat  
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14 7 is transferred mainly by radiation, while air circulation is used in the convection oven.  
15  
16 8 Compared to the situation in the deck oven, heat transfer is accelerated in the convection  
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18 9 oven, which results in faster drying of the product surface and thus in facilitating acrylamide  
19  
20 10 formation. Therefore, acrylamide levels in bread baked in a convection oven are usually  
21  
22 11 higher than those in bread originating from conventional baking processes (Claus et al.  
23  
24 12 2006). In model systems with cakes of wholemeal wheat, and wholemeal rye, it was  
25  
26 13 concluded that acrylamide production was maximized between 25 and 30 minutes, followed  
27  
28 14 by a slow linear reduction. Acrylamide did not occur to a large degree until the moisture  
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30 15 contents of the cakes fell below 5% (Elmore et al.2005).

31  
32  
33 16 • Breakfast Cereals

34  
35 18 Glycine can reduce the acrylamide content of a breakfast cereal although at higher  
36  
37 19 concentrations a very bitter taste develops (Lagrange J. and Vickers C., 2006 personal  
38  
39 20 communication). Acceptable tasting flakes could be made with a reduction of up to half or  
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41 21 three quarters of acrylamide. However the toaster operation had to be radically altered. It is  
42  
43 22 known that glycine gives rise to the carcinogen formaldehyde on heating (Low et al. 2006  
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45 23 and Davies and Wedzicha 1994); it may be that this is so volatile (bp -19.3°C) that it is lost  
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47 24 in processing. However, further work is required to assure that this is the case when it  
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49 25 pervades a food rather than being a surface treatment. Figure 3 shows the effect of glycine  
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51 26 addition on acrylamide formation.

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[Insert figure 3 about here]

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2 1 Wheat flakes were made on a pilot scale, those with more than 3g glycine per kilo dry  
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4 2 weight of ingredients were unacceptably bitter.

5  
6 3 We should not lose sight of the effect of the choice of grain. Wheat is the dominant  
7  
8 4 grain and dominates in bread and biscuit. The experience of sectors, which use other grains,  
9  
10 5 is enlightening. Figure 4 shows the acrylamide content of a breakfast cereal made to the  
11  
12 6 same process but from four different grains.

13 7 **[insert figure 4 about here]**

14  
15 8 One cannot substitute rice or oats for wheat and hope to have the same food.  
16  
17 9 However, figure 4 makes the point that wheat stands out from the other common grains. It  
18  
19 10 prompts the suggestion that if the agronomists could develop wheat that contained no more  
20  
21 11 asparagine than rice then more would be achieved than by any other measure presently  
22  
23 12 available. It is certainly desirable that plant breeders monitor new varieties in development to  
24  
25 13 assure that they do not inadvertently increase their asparagine content.

26 14 Fredriksson et al. (2004) have reported that the germ and the bran of wheat contain  
27  
28 15 more asparagine than the endosperm. Thus the use of refined flour would be expected to  
29  
30 16 yield less acrylamide than wholemeal flour. Industrial experience in this area indicates the  
31  
32 17 difficulty of writing general rules about recipe and process. One breakfast cereal  
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34 18 manufacturer recently adapted its extrusion-puff recipes to contain up to 25% wholegrain  
35  
36 19 wheat. The change was assessed for potential impact on acrylamide formation. The results  
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38 20 in table 3 show that wholemeal does not in all circumstances give rise to more acrylamide.  
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40 21 Results were also provided by a manufacturer who had experimented with wholegrain  
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42 22 biscuits; in this case wholemeal does give rise to more acrylamide. The difference may arise  
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44 23 from the fact that the mode of extrusion used creates gelatinised starch but with relatively  
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46 24 low thermal input and there is little maillard reaction. Such products are very pale unless  
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48 25 cocoa or one of the permitted yellow or brown colours is used. In contrast the biscuit was  
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50 26 made by a high temperature short time process that generates a significant brown maillard  
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52 27 colour.

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28 **[insert table 3 about here]**

1  
2 1 There are many processes in use and even within a process e.g. extrusion puffing  
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4 2 there are many variations in how it is affected. Thus some manufacturers extrude at or near  
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6 3 final product moisture, others extrude at higher moisture and toast the product to the target  
7  
8 4 moisture.

9  
10 5 Many processes involve two cooking steps, a pressure-cooking of dough or grain  
11  
12 6 then toasting after the dough is formed into a flake or other form. Several fundamentally  
13  
14 7 different toasting processes are in use e.g. baking ovens, rotary toasters and fluidised bed  
15  
16 8 systems. Observations made by Cereal Partners Worldwide indicate that although most  
17  
18 9 acrylamide forms in toasting, there can be measurable amounts ( $50\text{-}100\ \mu\text{g kg}^{-1}$ ) formed in  
19  
20 10 at moisture content from 10% to the low 30s%. It should not be assumed that acrylamide  
21  
22 11 only forms at very low moisture content. That said, there is no doubt that most acrylamide  
23  
24 12 forms in the toaster. Manufacturers normally control the process to deliver a uniform and  
25  
26 13 tightly defined target colour. Too light a colour is associated with a higher moisture content,  
27  
28 14 weak or raw taste and shorter storage life. Too dark a colour is associated with complaints of  
29  
30 15 over-baked taste and, of course with too low a moisture content and reduced yield.

31  
32 16 A detailed study of oven baking was made because this platform lends itself better to  
33  
34 17 instrumental measurement in process than other types of toaster. Figure 5 shows the  
35  
36 18 influence of oven temperature on formation of acrylamide in a baked cereal product.

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**[insert figure 5 about here]**

20 The results in figure 5 clearly demonstrate that lower temperatures are associated  
21 with less acrylamide formation. The most interesting feature was that the system was  
22 already at the lowest temperature at which the product successfully bakes, it was set to this  
23 point some years ago as part of a project to minimise energy consumption. Some other  
24 manufacturers have reported similar findings (personal communication). Simply reducing the  
25 temperature of an oven can be very costly for a manufacturer because the throughput is  
26 reduced and one may not be able to meet demand. Thus some points in figure 5 would  
27 reduce factory output to a third of normal. A more sophisticated approach reducing  
28 temperature but compensating by increasing airflow and adjusting humidity can avoid the

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1 problem of longer bake times. However, it requires an oven capable of such regulation and a  
2 scientific capability probably beyond the reach of smaller businesses.

3 A summary of major papers on mitigation of acrylamide in cereals and cereal  
4 products since 2004 is given in table 4.

5 **[insert table 4 about here]**  
6

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- 1  
2 1 • Impact of mitigation on the possible formation of other of other process contaminants.  
3  
4 2

5  
6 3 While strategies to reduce all process-derived contaminants based on temperature  
7  
8 4 moderation are well defined (Arrhenius temperature dependence), i.e. reducing temperature  
9  
10 5 will reduce acrylamide, the impact of recipe interventions on all known contaminants is not  
11  
12 6 clear. For example, investigations have shown that extended yeast fermentation  
13  
14 7 (Frederickson 2004; Hamlet et al. 2005a), pH reduction (Hamlet et al. 2005b) and the  
15  
16 8 addition of sodium chloride or glycerol (FEI / BLL 2005) can individually reduce acrylamide  
17  
18 9 formation in baked cereal products. However, all of these interventions can also lead to  
19  
20 10 significant increases in the generation of the process contaminant 3-MCPD in cereal  
21  
22 11 products (Hamlet et al. 2004; Hamlet et al. 2005c). An example of the effects of changing  
23  
24 12 dough pH on both acrylamide and 3-MCPD can be seen in figure 6.

25 13 **[insert figure 6 about here]**  
26 14

- 27 15 • Impact on nutrition.  
28 16  
29 17

30  
31 17 It is known that asparagine is naturally concentrated in the bran and the germ of  
32  
33 18 wheat (Taeymans et al. 2004). One might be concerned that this could give rise to increased  
34  
35 19 levels of acrylamide in wholegrain or bran enriched products. However, there is much  
36  
37 20 science that testifies to the health benefits associated with these products e.g. from cereal  
38  
39 21 antioxidants and fibre. Many epidemiological studies demonstrate that diets richer in  
40  
41 22 wholegrain or fibre are associated with benefits such as reduced risk of cardiovascular  
42  
43 23 disease, type 2 diabetes and overweight (Slavin 2004, Pereira et al. 2004). Intervention  
44  
45 24 studies are in progress to identify the causality of the effects and work such as the European  
46  
47 25 Healtgrain projects ([www.healthgrain.org](http://www.healthgrain.org)) seeks to identify the specific chemical entities in  
48  
49 26 bran and germ that drive the benefit. Careful consideration of the evidence for a significant  
50  
51 27 overall benefit of fibre and wholegrain is needed should we consider moving to more refined  
52  
53 28 (lower bran) flours to reduce acrylamide. Further work such as that reported in table 3

1 demonstrates that unrefined flour does not invariably lead to a higher acrylamide  
2 concentration. Statutory (Statutory Instrument (SI 1998)) or additional fortification of flours  
3 with sodium chloride (FEI / BLL 2005), divalent metal ions (Hamlet et al. 2005b) and the  
4 replacement of ammonium raising agents by sodium raising agents (Amrein et al., 2004)  
5 have been beneficial in reducing acrylamide in some bakery products. For example, figure 7  
6 shows the effect of divalent metal ion additions on acrylamide generation in wheat bread  
7 doughs. However, these metals are also important micronutrients and the impact on nutrition  
8 from increasing e.g. calcium levels to reduce acrylamide formation will need careful  
9 consideration.

10 **[insert figure 7 about here]**

- 11 • Impact on Maillard reaction products (MRP).

12  
13 In addition to the colours, tastes, and odours desired by producers and consumers of  
14 cereal products alike, other positive effects such as antioxidant activity (AOA) have also  
15 been attributed to MRPs. Some of these MRPs have beneficial effects on human health  
16 (Lindenmeier et al. 2002, Nicoli et al. 1997, Yen et al. 2005) as well as contributing to the  
17 shelf life of cooked products (Manzocco et al. 2000). Consequently strategies to reduce  
18 acrylamide formation by temperature and recipe modifications e.g. limiting reducing sugars,  
19 can impact significantly on AOA (Suma et al. 2006).

20 *Future outlook.* Our objective should be to reduce acrylamide without compromising the  
21 safety (toxicological), quality (organoleptic properties) and nutritional aspects of cereal  
22 products and to achieve this, it will be necessary to engage experts from all these areas.

#### 23 24 *Agronomic factors*

25  
26 One promising approach to reduce acrylamide in cereals products is to reduce the  
27 amount of asparagine that can react with reducing sugars. While investigations into the use  
28 of the enzyme asparaginase have shown a good potential for this purpose in some cereal

1  
2 1 products, little is known about the factors that affect the natural variations in asparagine  
3  
4 2 levels, both within and between cereal cultivars (Hamlet et al. 2005a). As we approach the  
5  
6 3 fifth crop year since the acrylamide issue, asparagine data for varieties of wheat, mill  
7  
8 4 fractions and other grains is still sparse.  
9  
10 5

11 6 *Future outlook.* More analytical effort is required to understand the apparent seasonal  
12  
13 7 variation in asparagine, the effect of milling interactions and the impact of environmental  
14  
15 8 factors on asparagine levels. There is a need to develop low asparagine cereal varieties that  
16  
17 9 retain the desired yield, milling and baking characteristics. Consequently, breeding programs  
18  
19 10 are needed, including the application of marker assisted breeding, which, in some markets,  
20  
21 11 will raise an interesting debate concerning acrylamide and genetically modified organisms.  
22  
23 12 An early dialogue with all crop breeders in conjunction with DG Agriculture should also be  
24  
25 13 established to ensure that accidental increases in asparagine levels do not result from  
26  
27 14 current breeding programmes.  
28  
29 15

30 16 *Recipe factors*

- 31 17  
32  
33 18 • Yeast.

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35 19  
36 20 Bakers yeast (*Saccharomyces cerevisiae*) can selectively consume asparagine and trials  
37  
38 21 with model breads have shown reductions of up to 80% for a proof time of 60 min (Hamlet et  
39  
40 22 al. 2005b). Investigations with yeast variants have shown that: a) asparagine consumption is  
41  
42 23 proportional to yeast addition level; and b) the rate of consumption of asparagine is  
43  
44 24 equivalent for both yeast low and high gassing yeasts (see figure 8). Consequently it should  
45  
46 25 be possible to optimise for both yeast gassing and asparagine reduction and this behaviour  
47  
48 26 should be explored further with yeast manufacturers.

49 27 **[insert figure 8 about here]**

- 50  
51 28 • Asparaginase.

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3  
4 2 Removal of asparagine by the degradation of the amide moiety can be accomplished by the  
5  
6 3 use of the enzyme asparaginase, which catalyses the hydrolysis of Asn to asparatic acid . In  
7  
8 4 fact, L-asparaginase is widely used for the treatment of leukaemia (Friedman et al. 2003)  
9  
10 5 and its therapeutic basis exploits the greater dependence of the malignant cells on an  
11  
12 6 exogenous source of Asn compared to normal cells. Zyzac et al. (2003) demonstrated that  
13  
14 7 asparaginase pre-treatment of a potato product could produce an Asn reduction of 88%, and  
15  
16 8 a corresponding acrylamide reduction of greater than 99% in the cooked material. Although  
17  
18 9 laboratory trials have been encouraging for bakery products, commercial scale trials are  
19  
20 10 required to demonstrate the effectiveness of asparaginases, particularly in low moisture  
21  
22 11 products such as biscuits types. Since one of the known minor side effects of clinical  
23  
24 12 asparaginase treatment is allergenicity, an evaluation of the safety of treated foods will be  
25  
26 13 required. Commercial food grade asparaginases are expected to be available shortly  
27  
28 14 although regulatory approvals may be prolonged. When and if it is accepted there is a good  
29  
30 15 reason to believe that the enzyme could allow "standard" recipes to be used so that the  
31  
32 16 sensory quality of the food is unchanged.

33 17  
34 18 *Process factors: From laboratory trials to commercial production.*

35 19 Ways to reduce acrylamide are often demonstrated on a laboratory scale, but they also need  
36  
37 20 to be feasible in a commercial environment.

38 21 Sadd and Hamlet (2005) demonstrated that acrylamide formation is very sensitive to  
39  
40 22 small changes in moisture, particularly at the lower range of moisture contents. Furthermore,  
41  
42 23 changes to mitigate for acrylamide e.g. increasing moisture and decreasing temperature  
43  
44 24 were also favourable for reducing other processing contaminants such as a 3-MCPD (figure  
45  
46 25 9). Although optimisation of moisture control can achieve a 15-20 % reduction in acrylamide  
47  
48 26 in laboratory trials, this does not necessarily carry over into an industrial production  
49  
50 27 environment where commercial ovens may not have the necessary degree of control.

1  
2 1 Although yeast has the potential to reduce asparagine in fermented dough, trials have  
3  
4 2 shown that this does not necessarily equate to less acrylamide in the cooked product. When  
5  
6 3 the yeast-fermented doughs were baked to generate acrylamide the corresponding reduction  
7  
8 4 in acrylamide was less than expected due to the release of fructose by yeast early in the  
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10 5 fermentation cycle (Hamlet et al. 2005a). Furthermore the addition of bakery fat also reduced  
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12 6 the effectiveness of asparagine removal by yeast (Hamlet et al. 2005b) indicating that this  
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14 7 application may not be effective in high fat products such as biscuits. Dough is complex and  
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16 8 dynamic system and further work will be required to understand and optimise processes to  
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18 9 minimise acrylamide.

19 10 [insert figure 9 about here]  
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21 11

## 22 12 **Conclusions**

23  
24 13 There are a limited number of successes for particular products. They are achieved  
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26 14 in the areas of recipe and processing. There are some promising leads for future, for  
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28 15 example asparaginase. We need more involvement of agriculture not only in hope of  
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30 16 developing crops, but also to avoid steps backward from the crops (asparagine is going up  
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32 17 and down). Involvement of experts in nutrition could help assure mitigation steps would not  
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34 18 cause an inverse negative effect in diet. Finally, we need a mechanism to assure that  
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36 19 mitigation of acrylamide does not enhance other process contaminants.

37 20 Cereal foods represent a very large and diverse range of raw materials and processes and it  
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39 21 is likely that each product / technology will require individual optimisation.

40 22 While we await the conclusions from further toxicological studies it is important that the  
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42 23 cereals sector continues to take the issue of acrylamide seriously and proactively  
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44 24 investigates how to reduce acrylamide in affected products. Regardless of the acrylamide  
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46 25 issue, cereals play an important role in the diet. In the meantime, the general advice to  
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48 26 consumers should be to continue to eat a varied diet composed of foods from many sources  
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50 27 and with moderate consumption of fried and fatty foods.  
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**Tables**

Table 1: The effect of the seasons on acrylamide levels of two breakfast cereals made by different processes but using the same wheat supply. Application of the t-test to the pooled data confirms that 2004 is different from 2003 and 2005 ( $P < 0.05$ ).

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Mean Acrylamide Levels in $\mu\text{g}/\text{kg} \pm 95\% \text{ CI}$		
Year of Harvest	A	B
2003	$117 \pm 24$ (n = 4)	$212 \pm 21$ (n = 4)
2004	$85 \pm 22$ (n = 5)	$145 \pm 47$ (n = 5)
2005	$145 \pm 33$ (n = 6)	$253 \pm 71$ (n = 6)

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Table 2. Monitoring results of acrylamide in Dutch spiced cake available on the Dutch market.

Year	Number of samples	Acrylamide $\mu\text{g kg}^{-1}$ Mean	Acrylamide $\mu\text{g kg}^{-1}$ Range
2002	13	1018	260-1410
2003	10	796	300-1480
2006	15	353	271-521

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Table 3. Effect of adding wholegrain to recipes. The number analysed refers to the number of trials with distinct batches of raw materials. (The results for breakfast cereal are from trials made by Cereal Partners Worldwide. Those for biscuit were provided by a biscuit manufacturer).

	Wholegrain %	Acrylamide ug kg <sup>-1</sup>	Standard deviation ug kg <sup>-1</sup>	Number analysed
Cereal 1	0	121	24	8
Cereal 1	25	114	25	7
Cereal 2	0	69	1	5
Cereal 2	12	44	7	6
Biscuit	-	75 - 302		4
Biscuit	+	1144 - 1275		4

Table 4 Summary of major papers on mitigation of acrylamide (AA) in cereals and cereal products since 2004.

Application	Outcome	Reference
Recipe for crackers	Asparaginase decreased AA by 70%. Replacement of ammonium carbonate by sodium hydrogencarbonate and reducing sugars by sucrose decreased AA with 80%. Decreasing free asparagine and reducing sugars in ingredients + lower temperature during baking decreased AA by 50%.	Amrein et al. 2004
Acrylamide in model systems	Water management may be key factor in controlling levels in foods.	Sadd and Hamlet, 2005 Ciessarová et al. 2006
Acrylamide in cracker model	Use of NaHCO <sub>3</sub> eliminated acrylamide. To lesser extent, NH <sub>4</sub> HCO <sub>3</sub> , cysteine, sodium bisulfite and ascorbate enhanced elimination. Some ingredients: citric acid, ferulic acid, and NaCl were found to decrease AA produced but had no effect on elimination.	Levine et al. 2005
Fermentation of dough	Reduction of free asparagine (asn) in dough by (longer) fermentation time resulted in breads with reduced AA.	Fredriksson et al. 2004 Hamlet et al. 2005a
AA in yeast-leavened wheat bread	Added asn increased content of AA, while added fructose did not influenced content. Temperature and baking time increased AA content in crust. Added asn did not influence colour.	Surdyk et al. 2004

Table 4 Continued...

Asparagine and sugars variation in wheat cultivars. Effects of processing & fermentation in breads.	AA was proportional to added asn. Asn variation was measured in eight cultivars: asn was as variable within as between. Sugars showed less variation and fructose had the most impact on acrylamide. Yeast fermentation decreased asn & hence AA, but the effect was offset by increased fructose release by yeast.	Hamlet et al. 2005a
Critical factors and possible reduction of AA in gingerbread	Ammonium hydrogencarbonate enhanced AA formation. Use of hydrogencarbonate reduced AA formation by >60%. Free asn was limiting factor, but AA can also be lowered by replacing reducing sugars by sucrose.	Amrein et al. 2004
Reducing AA in bakery products	Review of occurrence of AA in bakery products and recommendations for minimizing levels including agronomical, recipe and processing parameters.	Claus et al. 2006
Influencing AA content in rye crisp bread	Baking time and temperature increased AA. Added asn had significant effect on formation of AA, but fructose didn't. Added oat-bran with high content of mixed-linkage $\beta$ -glucan did not influence the AA content in breads.	Mustafa et al 2005
AA in wheat and rye model systems	AA formation maximized between 25-30 min of cooking, followed by a slow linear reduction.	Elmore et al. 2005
AA of semi-finished biscuit on industrial scale	Mitigation in industry-scale based on the optimization of baking agent, reducing sugars, and organic acid is feasible and compliant to high-quality standards.	Graf et al. 2006
Glycine reduces AA content in cereal products	Addition of glycine to dough resulted in 50->90% AA reduction in bread crusts. In flat breads, reduction varied between 60 and >95%.	Bråthen et al. 2005

**Figure captions**

Figure 1. Minimum reaction scheme used to model acrylamide formation a bakery products  
(adapted from Sadd and Hamlet 2005; Wedzicha et al. 2005)

Figure 2. The effect of recipe changes on the acrylamide content of wheat crackers

Figure 3. The effect of glycine on acrylamide formation. (Lagrange J. and Vickers C of  
Cereal Partners Worldwide)

Figure 4. The acrylamide content of breakfast cereal made to a common recipe and process  
but with different grains. (The experiments were made by Besson A. and Byrne S. in trials by  
Cereal Partners Worldwide).

Figure 5. The influence of oven temperature on formation of acrylamide in a baked cereal  
product. Points in red indicate that the product tasted raw; in blue that the baked taste was  
acceptable. (The experiments were made by Cereal Partners Worldwide in conjunction with  
Thistle Consulting and Prof. D. Mottram of the Department of Food Science ar Reading  
University).

Figure 6. Effects of initial dough pH on acrylamide and 3-MCPD generation in wheat bread  
doughs.

Figure 7. Effect of divalent metal ion additions on acrylamide generated in wheat bread  
doughs.

Figure 8. Effects of high (control) and low gassing rate bakers yeasts on asn levels in  
yeasted wheat bread doughs.

Figure 9. Effects of moisture and temperature on acrylamide (solid contours) and 3-MCPD  
(dashed contours) generated in wheat doughs. Data from: Sadd and Hamlet 2005; Hamlet et  
al.2004.

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**Figures.**

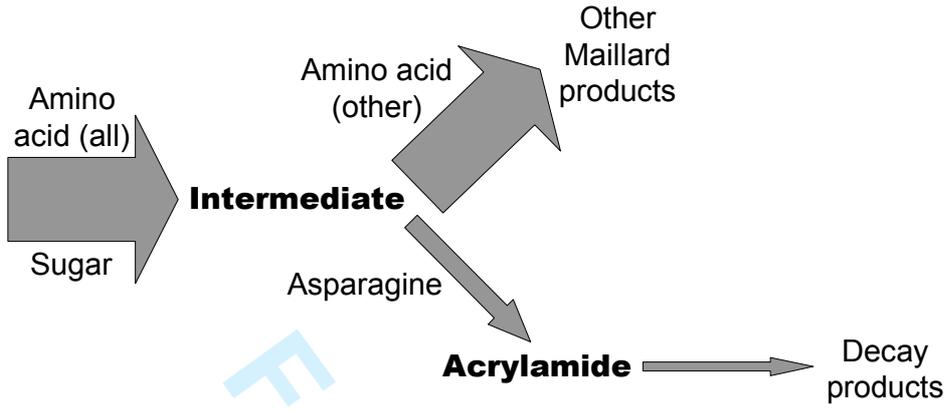


Figure 1.

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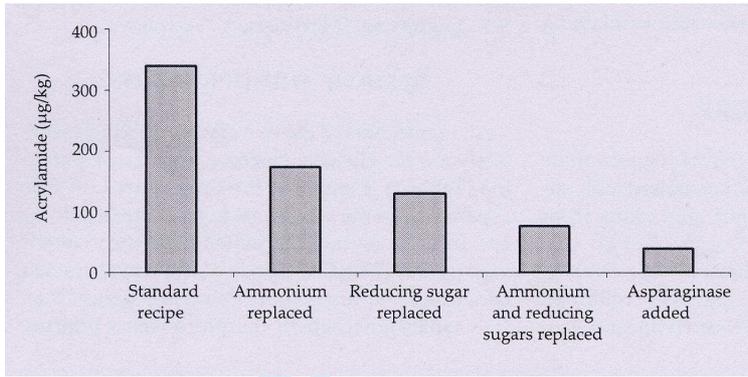


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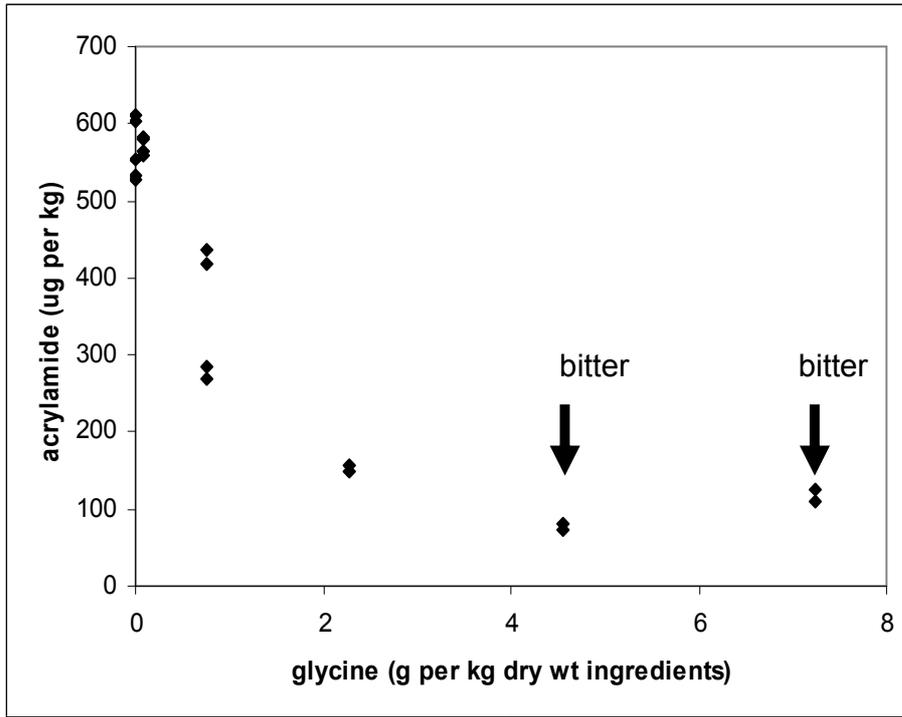


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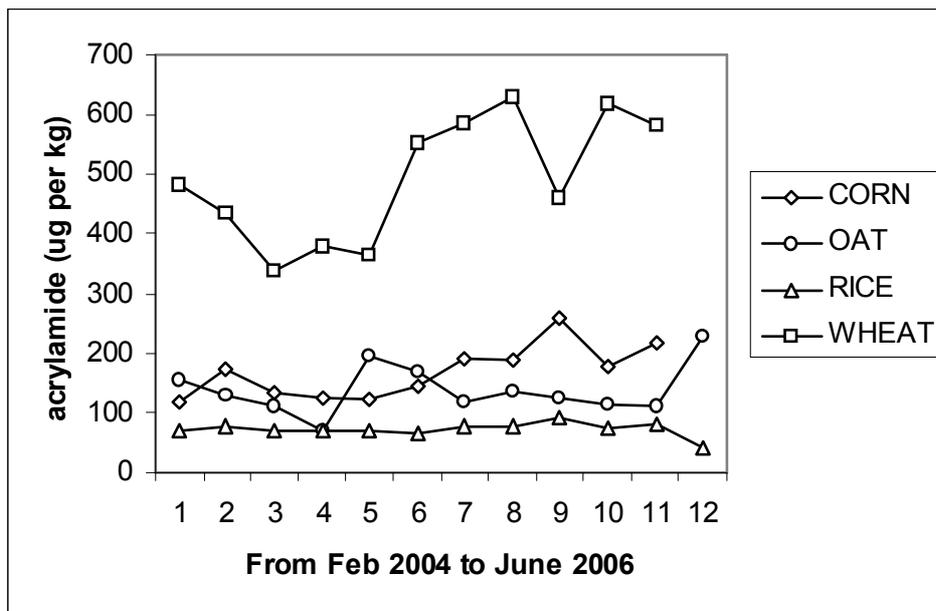


Figure 4

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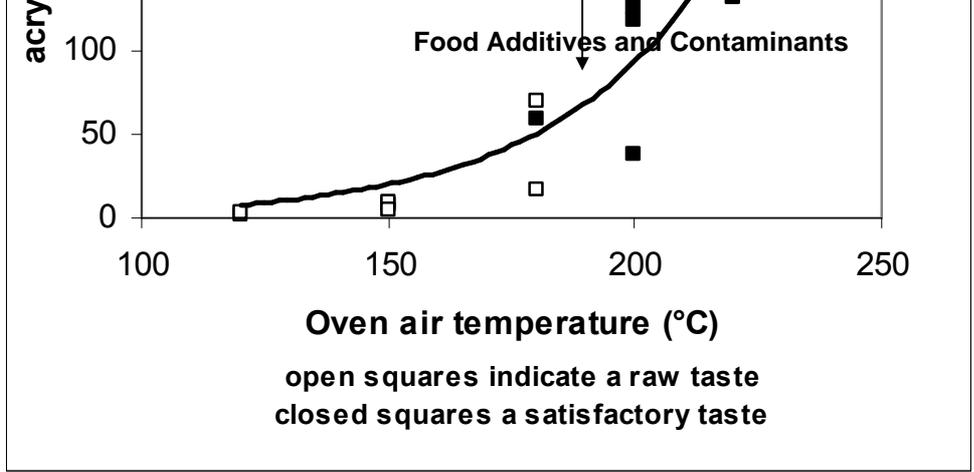


Figure 5.

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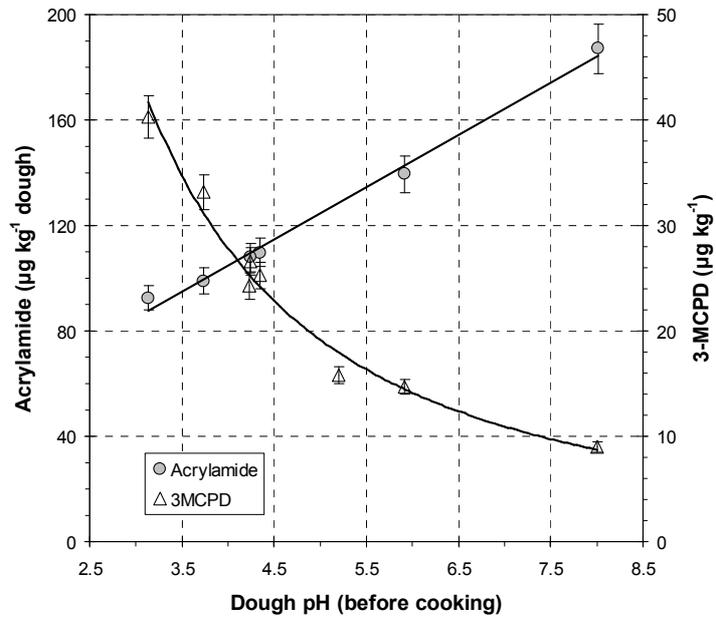


Figure 6.

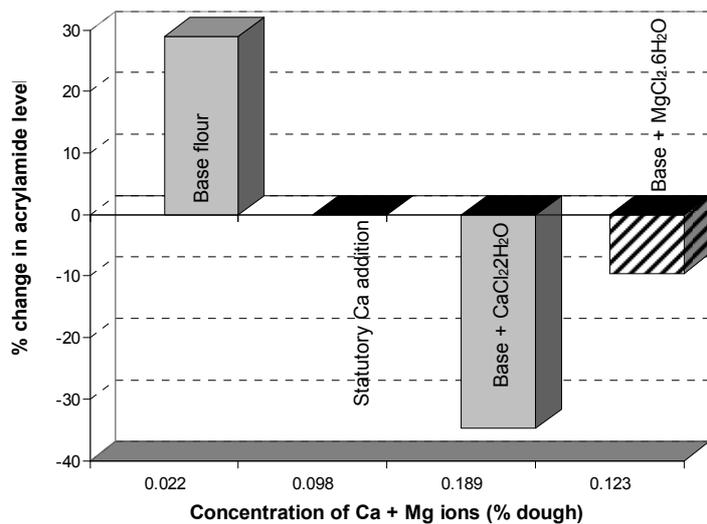


Figure 7.

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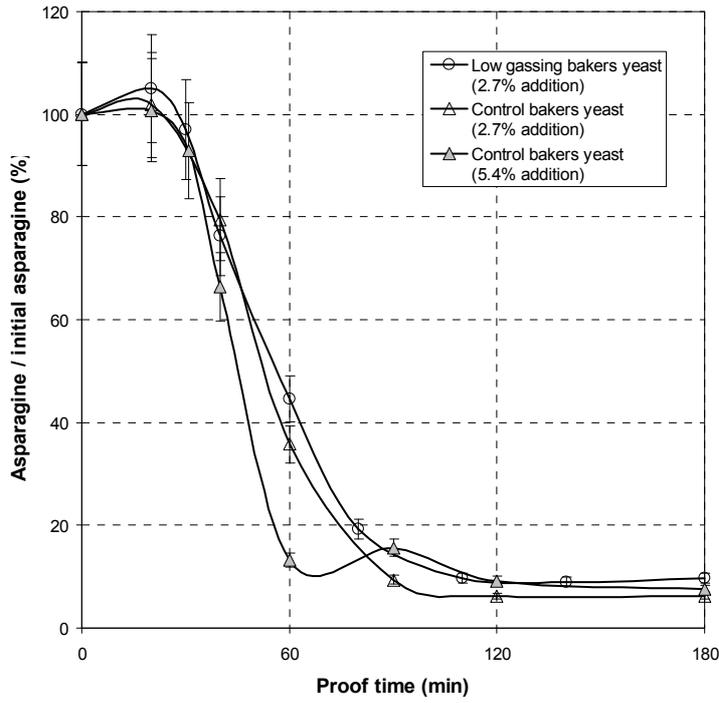


Figure 8.

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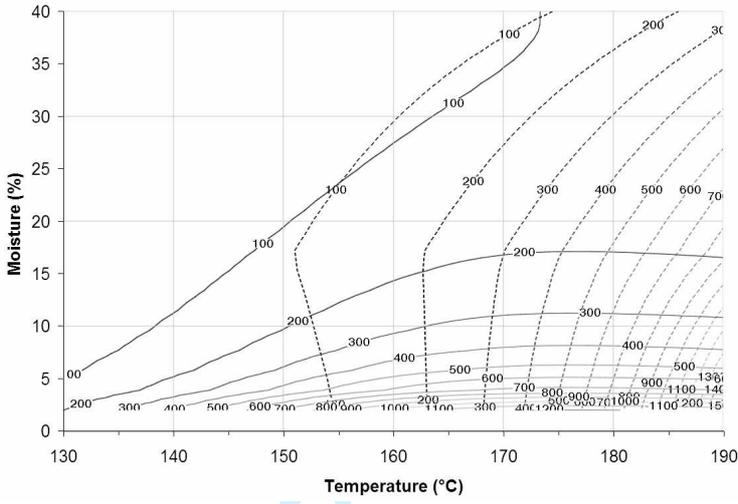


Figure 9.

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