The CIAA Acrylamide "Toolbox"

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Key Changes to the CIAA Acrylamide Toolbox
Status December 2008

Grocery Manufacturers Association (GMA) information integrated into the CIAA Toolbox; this entails endorsement and review activities of GMA and in essence marks the progression toward a “global” acrylamide Toolbox.

The latest sectorial reviews (CAOBISCO, UEITP and ESA) on the usage of the tools stipulated in the Toolbox have been included.

Information from Switzerland on sugar contents in potatoes have been added.

“Recipe: Raising agents (e.g. ammonium and sodium salts)” modified to better reflect the content of the tool.

“Processing: Asparaginase” is now listed as a separate tool.

Latest scientific publications (e.g. risk-benefit modelling on sodium exposure) and project updates (e.g. BLL) included where relevant.

The CIAA Acrylamide "Toolbox"

Summary

The CIAA "Toolbox" reflects the results of several years of industry cooperation to understand acrylamide formation and potential intervention steps. Its aim is to provide brief descriptions of the intervention steps evaluated and, in many cases, already implemented by food manufacturers and other partners in the food chain.

New in this latest edition is the inclusion of information from food and beverage manufacturers in the USA, provided through the Grocery Manufacturers Association (GMA). GMA will in future be actively involved in providing information on mitigation measures and be integrated in the Toolbox revision and final validation processes. This corroborates the global applicability and use of the Acrylamide Toolbox.

The Toolbox approach is intended to assist individual manufacturers, including small and medium size enterprises with limited R&D resources, to assess and evaluate which of the intervention steps identified so far may be helpful to reduce acrylamide formation in their specific manufacturing processes and products. It is important that they assess the suitability of proposed mitigation steps in the light of the actual composition of their products, their manufacturing equipment, and their need to continue to provide consumers with quality products consistent with their brand image and consumer expectations. It is anticipated that some of the tools and parameters will also be helpful within the context of domestic food preparation and in food service establishments, where stringent control of cooking conditions may be more difficult.

A total of 14 parameters, grouped within the four major Toolbox compartments, have been identified. These parameters can be applied selectively by each food producer in line with their particular needs and product/process criteria. In addition, the stage at which the different studies have been conducted, i.e. laboratory, pilot, or in a factory setting (industrial), are aligned to the potential mitigation measures. This approach ensures that all pertinent tests and studies are captured independent of their immediate applicability to commercial production.

The Toolbox is not meant as a prescriptive manual nor formal guidance. It should be considered as a "living document" with a catalogue of tested concepts at different trial stages that will be updated as new findings are communicated. Furthermore, it can provide useful leads in neighbouring sectors such as catering, retail, restaurants and domestic cooking. The final goal is to find appropriate and practical solutions to reduce the overall dietary exposure to acrylamide. The latest version of the toolbox can be found at: www.ciaa.be.

To assist SMEs in the implementation of the Toolbox, CIAA and the European Commission, Directorate General Health and Consumer Protection (DG-SANCO) in collaboration with national authorities developed the *Acrylamide Pamphlets* for five key sectors: Biscuits, Crackers & Crispbreads, Bread Products, Breakfast Cereals, Fried Potato Products such as Potato Crisps and French Fries. Individual operators can use the tools outlined in the pamphlets to adapt their unique production systems.
The pamphlets are available in > 20 languages on the following website:
http://ec.europa.eu/food/food/chemicalsafety/contaminants/acrylamide_en.htm

Background

In April 2002, authorities, food industry, caterers and consumers were surprised by the unexpected finding that many heated foods contained significant levels of acrylamide, a substance known until then only as a highly reactive industrial chemical, present also at low levels for example in tobacco smoke. The toxicological data suggested that this substance might be – directly or indirectly – carcinogenic also for humans. Recent assessments by JECFA, WHO and SCF confirmed that such a risk cannot be excluded for dietary intake of acrylamide, but did not confirm that this would be relevant at the low dietary exposure level compared to other sources of exposure, e.g. occupational. At the EU level, progress in the research on acrylamide has been shared openly and regularly through forums such as the "Acrylamide Stakeholder meetings", held in October 2003 and January 2005. A formal presentation was given to the Commission Working Group on 16 September 2005, where the Commission and national authority representatives supported the Toolbox concept: CIAA was urged to publish the first official version of the Toolbox without delay. The present text is the third edition incorporating latest developments and knowledge, including the key points that were presented at the joint CIAA/EC Workshop on Acrylamide held in Brussels in 2006 [1]. Furthermore, there has been an active and very close contact between the CIAA and HEATOX, and the outcome of the HEATOX project in relation to advice for industry and the catering sector is comparable with the Toolbox content. To avoid duplication of efforts, the results generated by HEATOX and relevant to industry/catering have been fed into the CIAA Toolbox (*HEATOX Final Report,* 12 April 2007, www.heatox.org).

It has been confirmed that a wide range of cooked foods – prepared industrially, in catering, or at home – contain acrylamide at levels between a few parts per billion (ppb, μg/kg) and in excess of 1000 ppb. This includes staple foods like bread, fried potatoes and coffee as well as speciality products like potato crisps, biscuits, crisp bread, and a range of other heat-processed products.

It is now known that acrylamide is a common reaction product generated in a wide range of cooking processes, and that it has been present in human foods and diets probably since man has cooked food.

Immediately following the initial announcement, the food industry within the EU took action to understand how acrylamide is formed in food, and to identify potential routes to reduce consumer exposure. From the onset of the acrylamide issue, the efforts of many individual food manufacturers and their associations have been exchanged and coordinated under the umbrella of the European Food and Drink Federation (CIAA), to identify and accelerate the implementation of possible steps to reduce acrylamide levels in foods. These efforts are also intended to explore how the learnings developed by industry might also be applied in home cooking and catering which contribute to more than half of the dietary intake of acrylamide.

[1] The proceedings of the Workshop are published in a Supplement of *Food Additives and Contaminants* vol. 24 (S1), 2007.

Most of the tools described in this document relate to what is now seen as the main formation mechanism of acrylamide in foods, i.e. the reaction of reducing sugars with free asparagine in the context of the Maillard reaction. In fact not only sugars but also reactive carbonyl compounds may play a role in the decarboxylation of asparagine – a necessary step in the generation of acrylamide. Several intermediates in the “dry” Maillard reaction cascade have been proposed, such as 3-aminopropionamide (3-APA). The role of this compound as a key transient intermediate has recently been confirmed through isotope labelled experiments in a cheese model (*J. Agric. Food Chem.* (2006) 54: 5933-5938). Very recent work has shown that acrylamide may also be found in relatively high amount (> 1000 ppb) in foods not subjected to typical cooking temperatures, such as dried fruits (e.g. plums, pears). The drying conditions of these products are typically < 80°C, and thus 3-APA may also play a role in the formation of acrylamide during the drying process that can extend over a period of several days (*Food Addit. & Contam.* (2007) 24:13-25).

Other pathways that do not require asparagine as a reactant have been described in the literature, such as acrolein and acrylic acid. The thermolytic release of acrylamide from gluten in wheat bread rolls was demonstrated as an alternative pathway (*Mol. Nutr. Food Res*. (2006) 50: 87-93). Based on molar yields, these mechanisms can be considered as only marginal contributors to the overall acrylamide concentration in foods.

In many cooking processes, the Maillard cascade is the predominant chemical process determining color, flavour and texture of cooked foods, based on highly complex reactions between amino acids and sugars, i.e. common nutrients present in all relevant foods. The cooking process *per se* – baking, frying, microwaving – as well as the cooking temperature seem to be of limited influence. It is the thermal input that is pivotal: i.e. the combination of temperature and heating time to which the product is subjected. In some product types it has been found that the acrylamide content decreases during storage. This has been observed in packed roast coffees where it is based on a temperature-dependent reaction. Research indicates that acrylamide interacts with the coffee matrix by an as yet unknown mechanism (Baum,*et al.*, *Mol. Nutr. Food Res*. (2008) 52:600-608). A recent study also found a decrease in acrylamide during storage of milled unfermented rye crisp bread. Temperature and moisture content seemed to be important for the decrease (Mustafa *et al.*, *J. Agric. Food Chem*. (2008) 56(23):11234-11237). Further studies into the mechanisms behind these effects are warranted.

Both asparagine and sugars are not only important and desirable nutrients, naturally present in many foods, they are also important to plant growth and development. In most foods, they cannot be considered in isolation, since they are part of the highly complex chemical composition and metabolism of food plants. The Maillard reaction depends on the presence of a mixture of these common food components to provide the characteristic flavour, color and texture of a given product. Thus, most of the Maillard reaction products are highly desirable, including some with beneficial nutritional properties and health effects.

Consequently, any intervention to reduce acrylamide formation has to take due account of the highly complex nature of these foods, which therefore makes it very difficult to decouple acrylamide formation from the main Maillard process. The University of Leeds is developing a kinetic model to predict acrylamide formation under different processing conditions. The deliverable will be an “industry toolkit” to mitigate acrylamide in food (cereal based products and potatoes), and would ideally be integrated into the CIAA Toolbox once validation work has been completed.

It is essential to appreciate that elimination of acrylamide from foods is virtually impossible – the principal objective must be to try to reduce the amount formed in a given product. However, current knowledge indicates that for some product categories, what can be achieved is dependent on natural variations in raw materials.

Whilst the Toolbox can provide useful leads, its practical application in domestic cooking, and catering requires additional work.

Glycidamide Formation in Food

Glycidamide is the genotoxic metabolite of acrylamide and the potential formation of glycidamide in food has now been addressed. Researchers have shown that the epoxidation of acrylamide by fatty acid hydroperoxides - formed during lipid peroxidation - is another possibility for interactions of acrylamide with food constituents. Fatty acid hydroperoxides can mediate the formation of glycidamide from acrylamide, albeit in minute amounts (1.51 μg/kg in a single potato chip sample and 0.002 – 0.29 μg/kg glycidamide in light colored precooked French fries) and so far demonstated only in very selected foods (potato chips and fries). Based on this work, the potential burden of glycidamide via food is negligible (Granvogl *et al., J. Agric. Food Chem*. (2008) 56:6087-6092).

Reaction of Acrylamide with Food Constituents

It is well established that acrylamide may react with nucleophiles and recent work at laboratory scale has demonstrated the formation of acrylamide-cysteine adducts in cereal flours that were heated at 170°C for 20 min. However, no correlation between the formation of the acrylamide-cys adduct and the amount of free acrylamide and cysteine could be established (Granvogl *et al.,*in: "Development of new procedures for heated potato and cereal products with reduced acrylamide contents. BLL/FEI Report 2008, Bonn, pp 9-21).

Methods of Analysis and Sampling

Today, many laboratories offer sensitive and reliable methods to analyse acrylamide in a wide range of foods. Issues with the extractability of acrylamide in certain food matrices were raised by Eriksson & Karlsson, showing that a high extraction pH may significantly enhance the yield of acrylamide versus extraction under neutral pH conditions (*LWT-Food Science and Technology* (2006) 39: 392-398). This observation suggests that the methods developed to date may underestimate the concentration of acrylamide in certain foods. Work done by independent research groups (Goldmann *et al*., *Food Addit. & Contam.* (2006) 23:437-445, and Hajslova *et al*., presentation at Cost 927 Workshop, Napoli, 2006) confirmed, however, that the "additional" acrylamide released at high pH is not due to the improved extractability of the analyte from the food matrix, but rather an extraction artefact formed due to the decomposition - under extreme pH conditions - of certain hitherto unidentified precursors. Consequently, the choice reliable of analytical methods is of crucial importance.

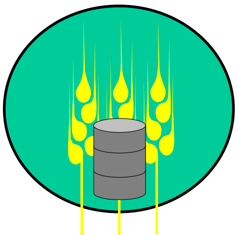
The main challenge for the analyst is the high variability of the products. This starts from the natural variability of a given raw material – any potato can be considered as "individual" with noticeable differences in composition and thus potential for acrylamide formation. Slight differences in product composition and process conditions, and even the location within the temperature range of one specific production line, may lead to major differences in acrylamide levels, often of several multiples between samples derived from the same product recipe made on the same production line.

Appropriate sampling and statistically relevant numbers of analyses are therefore essential to determine acrylamide amounts in products, and to assess the actual reduction achieved by the mitigation step(s) when conducted in a factory setting.

Definition and Use of the Toolbox Parameters

The summaries describing the various acrylamide reduction tools developed by industry are intended to be generic. It is necessary to take account of the differences between product recipes, designs of processes and equipment, and brand-related product characteristics even within a single product category.

The following 14 parameters, grouped within the original Toolbox compartments, have been identified:

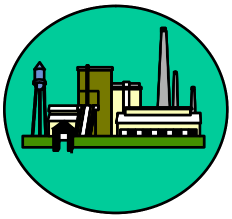


- Agronomical

- Sugars
- Asparagine

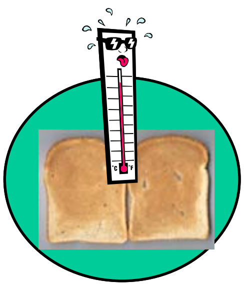
- Recipe

- Raising agents
- Other minor ingredients, e.g. glycine and divalent cations
- pH
- Dilution
- Rework



- Processing

- Fermentation
- Thermal input and moisture control
- Pre-treatment (e.g. washing, blanching, divalent cations),
- Asparaginase



- Final Preparation

- Color endpoint
- Texture/flavour
- Consumer guidance

However, it needs to be emphasised that **there is in most cases no single solution to reduce acrylamide in foods**, even in a given product category. Indeed, individual processing lines dedicated to the manufacture of the same product in one factory may need different applications of the proposed tools. As an example, modification of thermal input for comparable product quality can be achieved by frying at a lower temperature for an extended time span, or by "flash frying" for a very short time at higher temperatures. The choice will depend on the design and flexibility of the existing production equipment.

The summaries in this document also specify the level of experience available for a proposed intervention, i.e. trials conducted at (i) laboratory/bench, (ii) pilot, or (iii) industrial scale. Here, it is important to discern interventions tested at laboratory or pilot scale and those that have been assessed in industrial trials. To avoid confusion, a separate Table has been established that lists **only those tools that were found by manufacturers to work in their industrial settings** and may be applied either singly or in combination to mitigate acrylamide in commercial products (see **Annex**).

- **Laboratory Scale:** This indicates that - for the categories mentioned - only experimental work has been done to assess the impact of the proposed intervention. Most likely no quality tests (organoleptic, shelf-life studies, nutritional impact, etc.) have been conducted nor full assessment of the legal status or possible intellectual property rights for the given intervention. Large scale industrial application has either not yet been done or has failed in the specific context. This does not necessarily mean that the concept would not function for other applications.

- **Pilot Scale:** These concepts have been evaluated in the pilot plant or in test runs in the factory, but not yet applied successfully under commercial production conditions.

- **Industrial Scale:** These interventions have been evaluated and implemented by some manufacturers in their factories. Application by other manufacturers may or may not be possible depending on their specific process conditions. The validation of the suggested tools was assessed over the product shelf life. The legal status of the proposed measures has been evaluated.

Most of these tools have been evaluated only in the industrial, food processing context. Their usefulness for caterers or domestic cooking will need to be assessed separately, given the differences in cooking conditions and the typically lower level of standardisation and process control in non-industrial settings.

Where available, literature references are provided for the tool descriptions. In many cases, however, the summaries also include unpublished information provided by individual food manufacturers and sectors contributing to the joint industry programme coordinated by the CIAA.

The tools described do not comprise an exhaustive list of mitigation opportunities. The work of both industry and academic researchers continues and is likely to provide additional intervention leads or improvements. It is CIAA's intention to continuously update the Toolbox so as to reflect such developments.

Regulatory Compliance

Any intervention must also be evaluated for its regulatory impact. For many products, the use of additives is strictly regulated and changes in recipes will not only affect the ingredient list but potentially also the product name and description and customs classification. Additionally, process conditions and equipment standards must continue to meet relevant official standards. New potential ingredients or processing aids need to undergo regulatory approval, including any health and safety considerations. For new plant cultivars, success in breeding must be followed by formal approval of the new seed. All these considerations can influence the choice of interventions and the time to implementation/commercialisation.

In the case of the enzyme asparaginase, companies are today producing a commercial food-grade enzyme. As worldwide licensees for the patent holders, they may sub-licence the rights to food manufacturing and processing companies to incorporate asparaginase in their food production processes to lower the amounts of acrylamide.

GRAS status has been obtained from the US FDA for both types of asparaginases in the products of intended use. JECFA reviewed asparaginase from *Aspergillus oryzae* at its 68th meeting in June 2007, and on the basis of available data and total dietary intake arising from its use concludes that asparaginase does not represent a hazard to human health (*Joint FAO/WHO Expert Committee on Food Additives (JECFA): Report on 68th meeting, Geneva, 19-28 June 2007*). However, regulatory permission to apply asparaginase in foods requires clarification, nationally and internationally.
If products containing asparaginase are brought to the market it is important to check the regulatory status of asparaginase and of the products in that country, which includes EU Member States.

Risk/Risk and Risk/Benefit Positioning

Mitigation of acrylamide formation through changes in product composition and/or process conditions may have an impact on the nutritional quality (e.g. decreased nutrient bioavailability; changed flavour, taste/palatability, texture), and safety of food (e.g. inadequate reduction of microbial load, decomposition of natural toxins or inadvertent formation of other undesirable substances). There may also be potential loss of beneficial compounds generated during cooking which are known to have protective health effects, e.g. antioxidants and *in vitro* antioxidant capacity of heated foods, see: *J. Agric. Food Chem.* (2006) 54:853-859; *LWT- Food Science and Technology* (2007) 40:1849-1854*.*

- Frying potatoes at lower temperatures to a comparable endpoint can reduce acrylamide formation, but will require longer cooking times and can consequently increase the fat uptake (ref: industrial sources).
- Excessive blanching of potatoes results in further loss of minerals and vitamins.
- Using refined flour reduces acrylamide formation potential, but is seen as less nutritionally desirable compared with whole grain (bran) products.
- Replacing ammonium bicarbonate with sodium bicarbonate helps control acrylamide formation, but if applied systematically will increase sodium levels. Recently, a risk-benefit analysis has been conducted on increased sodium intake as a potential risk factor for cardiovascular disease against the (still putative) risk of acrylamide exposure. Mitigation of acrylamide in biscuits and ginger bread was accompanied by a small increase in sodium intake. Around 1.3 % of the population shifted from a sodium intake below to above 40 mg/kg bw/d (Seal *et al*., *British J. Nutr.* 99 (Suppl. 2): S1-S46, 2008.

Therefore, for any proposed intervention, a risk/risk or risk/benefit comparison should be conducted to avoid creating a potentially larger risk.

Other Considerations

- **Manufacturer Specificity:** Each manufacturer needs to explore how a proposed intervention can be implemented in its specific situation; especially when moving from laboratory experiments or pilot plant trials to routine production in the factory to ensure comparable results under commercial conditions.

- **Interactions between multiple interventions:** Often more than one intervention step may be applied (see **Annex 1**). These individual interventions may lead to an overall reduction of the desired mitigation effect. Particularly in products with highly complex recipes like biscuits, it is very difficult to predict the "real life" impact of a given measure.

- **Process Compatibility:** Any proposed intervention also needs to be assessed for its feasibility and ability to be integrated into an existing factory setting. For example, is space available for any additional storage tanks to add a new ingredient? Will changes affect the line speed and thus the output and competitiveness of a factory? Are new components compatible with the existing equipment, for e.g. the possible corrosive effects of food-grade acids.

- **Natural Variability:** Foods are based on natural commodities like cereals, potatoes or coffee beans. Their composition varies between crop cultivars, harvest season, climatic conditions, soil composition and agronomic practices. Properties also change with storage and initial processing, e.g. extent of milling. These differences and their impact on acrylamide formation are so far poorly understood and can thus not be consistently controlled. Seasonal and year-to-year variability of raw materials can have a greater impact on acrylamide levels than any of the interventions implemented, and must be taken into consideration.

- **Process Variability:** There is a significant variability in acrylamide levels between products of even a single manufacturer, in many cases even within one product range. Thus, to assess the impact of a given intervention, especially if multiple changes are made in parallel, a sufficient number of analyses are needed to permit comparisons: single analyses are nearly always insufficient to evaluate the effect of an intervention for a given product.

- **Brand Specific Consumer Acceptance:** Each manufacturer needs to assess the impact of the proposed interventions on its brand-specific product characteristics. A modified product may well appear acceptable in principle, but after the modification may no longer match the consumer's expectation for an established brand. Thus, improvement of an existing product, in terms of reduced acrylamide content, may be more difficult to achieve than in the case of a newly developed product.

Abbreviations used

AA: acrylamide; **Asn**: asparagine; **BLL**: Bund für Lebensmittelrecht und Lebensmittelkunde e.V.; **Caobisco**: Association of the Chocolate, Biscuits and Confectionery Industries of the EU; **EC**: European Commission; **ESA**: European Snack Association; **GAP**: Good Agricultural Practice; **GMA**: Grocery Manufacturers Association; **UEITP**: Potato Processors Association of Europe.

Agronomical: Sugars

Potato (Potato products for frying, roasting, baking, but mainly French fries and potato crisps)

Industrial scale

Minimising sugars has been part of standard manufacturing practice

Reducing sugars are one of the key reactants for the formation of AA [1]. The sugar content of the tuber correlates well with the AA concentration in the product especially if the fructose/Asn ratio is < 2 [2]. Controlling sugar is currently the primary measure employed by the industry to reduce AA levels in crisps and French fries [3] by:

Selection of potato varieties with low reducing sugars that are suitable for the product type is key.

Control of tuber storage temperature identified as good practice

- Lot selection based on reducing sugars content (crisp industry) or color assessment of a fried sample (French fry industry); good correlation between sugars content and color [4]
- Controlling storage conditions from farm to factory (e.g. temp. > 6°C identified as good practice for long storage [5], use of sprout suppressants following GAP, reconditioning at higher temp. (e.g. ambient) over a period of a few weeks).
- Ensure that tubers are mature at time of harvesting (immature tubers tend to have higher sugar levels).

These measures are implemented throughout the industry.

N-fertilisation may impact reducing sugar content

Future opportunities include:

- Breeding new potato varieties with lower reducing sugar content and/or less cold sweetening effect.
- Further optimise the agricultural practices to reduce sugars and Asn. The nitrogen fertiliser regime appears to influence the reducing sugar concentration of the potato tuber, i.e. increased reducing sugars (60 – 100%) upon lowering the field N-fertilisation [6].

Cereal (e.g. bread, crispbread, biscuits/bakery wares, breakfast cereals)

Pilot & Industrial scale

Sugars composition of cereal grains is not a key determinant of AA formation

Research confirms that Asn rather than sugars is the key determinant of AA formation in cereal products. The sugars composition of cereal grain has not previously been considered relevant to breakfast cereal manufacture.

Measurements for four soft wheat varieties in 2004 found 1 to 1.3% dry wt of total reducing sugars as glucose (0.41 - 0.58%); fructose (0.17 - 0.2%) and maltose (0.36 - 0.55%). Sucrose was at 0.5 - 0.65%. AA formation showed no relation to total reducing sugars or to individual sugars concentration. Recent work has shown a wide variation of reducing sugars in different cereal grains and their fractions, e.g. fructose/glucose amounts are highest in wholemeal rye/wheat. Furthermore, Asn and fructose/glucose were correlated in soft wheat and rye indicating that measurement of the latter sugars could also be used to select cereals (for reduced acrylamide formation) and may also be beneficial in reducing acrylamide in products that do not contain added reducing sugars in their recipe. No correlation was found for bread and wheat flours [7].

Coffee and Coffee Mixtures (e.g. roast and ground, soluble coffees, coffee mixtures)

Pilot scale No correlation to AA formation	**Coffee** Sugar levels in the green beans (robusta, arabica) show no correlation to the amount of AA formed during roasting. **Chicory** Inulin and sucrose at approx. 67g/100g dried chicory, and reducing sugars approx. 1.9g/100g (dry wt). These amounts increase substantially during roasting. No relationship between sugar levels and AA formation during roasting [8].

Other considerations

Potatoes:

- Effect of optimisation of the reducing sugar content in the raw materials on other components influencing nutritional properties.
- Minimising reducing sugar content needs to be balanced against processing methods and final product characteristics (color, flavour, etc.).

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Agronomical: Asparagine (Asn)

Potato (Potato products for frying, roasting, baking, but mainly French fries and potato crisps)	
Lab scale Currently no practical means available to control Asn level in potatoes	Asn, an important amino acid for plant growth, is the other key reactant for AA formation. In potatoes, Asn is the most abundant free amino acid, typically 0.2 - 4% dry wt, 20 - 60% of total free amino acids. Asn levels do not correlate to sugar levels. So far no control of Asn levels in potatoes has been established. Potential leads being explored include: • breeding of lower Asn varieties • impact of storage on free Asn levels Impact of farming practices (e.g. fertiliser regimes) on Asn levels. In potatoes, the effect of sulphur is uncertain, and any advice on S-fertilisation to farmers would be premature based on the studies conducted so far [1, 2].
Transformed intragenic plants with ca. 20-fold lower amount of Asn in the tuber	Genetically modified potato with silenced Asn synthase genes in the tuber have up to 20-fold reduced amounts of free Asn. Heat processed products (French fries and potato chips) derived from such tubers show comparable sensorial properties to their non-GM counterparts, and expectedly much lower levels of AA. This work is based on lab trials and the performance of the tubers in the field has not been assessed [3].
Cereal (e.g. bread, crispbread, biscuits/bakery wares, breakfast cereals)	
Pilot scale Selection of crop varieties with lower Asn could lower AA levels in finished products The importance of maintaining sulphur levels for cereal cultivation must be stressed to farmers	Asn is the critical component which leads to the formation of AA in cereal products [4]. Free Asn within and between cereal types varies widely [5]. Year to year variations from one harvest to another are considerable and more fundamental knowledge is needed concerning the impact of agronomical practices and cereal varieties on Asn level. Sulphur-deprived soils have been shown to impact the free Asn concentrations in certain cereal crops considerably [6]. Cooked wheat prepared from sulfur-deficient flour also impacts the spectrum of aroma compounds, and consequently the organoleptioc properties [7]. However, the very dramatic effects of extreme sulphur deprivation are not relevant because the crops are stunted. However it does appear that there is an effect at levels realistic in farming. Even a 25% reduction in asparagine would achieve more than most interventions tested to date. Choice of wheat with lower free Asn has led to products with lower AA levels [8]. The major soft wheat varieties in use in the UK are, perhaps by chance, those with lowest Asn concentration. In France, more variation is seen and selection of soft wheat varieties has been applied. After three years trial by one manufacturer it appears that environmental variation between growing sites is too large for such selection to work on a large industrial scale. Current experience suggests that specifying low Asn wholegrain is not yet possible, but that using less whole meal and more endosperm will be effective but will change the product's organoleptic and nutritional properties [9]. Crispbread: analysis of rye samples has shown that environmental factors have more influence than rye variety. It has not been possible so far to identify which environmental factors have the most significant effect.

Coffee and Coffee Mixtures (e.g. roast and ground, soluble coffees, coffee mixtures)

Pilot scale No mitigation options through crop selection due to narrow window of free Asn. Contribution of marginal pathways not yet clarified. Agronomic aspects not adequately studied and are considered long-term	**Coffee** Free Asn concentrations in green coffee beans lie within a very narrow range, typically from 20–100 mg/100g, and thus do not provide the opportunity for possible control or reduction by selection of beans with relatively low amounts of free Asn. On average a tendency of slightly higher AA content of roasted Robusta beans have been reported which in some cases may reflect the concentrations of Asn in the green coffee beans [10, 11]. As identified during the CIAA/EC Workshop [12,13], modelling studies of AA formation in coffee will be important to understand to what extent Asn is a key reactant and the potential contribution of minor pathways (e.g. thermolytic protein cleavage) in this product category. **Chicory** The range of free Asn in chicory roots is relatively narrow (40 - 230 mg/100g). Studies at pilot scale show that Asn content of dried chicory is correlated to the formation of AA [8].

Other considerations

Impact of reducing free Asn on plant health and consumer nutrition.
In coffee, other "marginal" pathways not related to free Asn may become important for the formation of AA [10,12].

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Recipe: Raising agents

Potato (Potato products for frying, roasting, baking, but mainly French fries and potato crisps)

Not applicable

Cereal (e.g. bread, crispbread, biscuits/bakery wares, breakfast cereals)

Pilot & Industrial scale Reducing or replacing NH_4HCO_3 in recipes, which is now used in certain commercial applications is an option to lower AA, but the impact on organoleptic properties must be assessed	**Biscuits** Replacing NH_4HCO_3 with alternative raising agents is a demonstrated way to relatively lower AA in certain products and on a case-by-case basis [1]. Despite changes to flavour, color and texture, several products (sweet biscuits and gingerbread) have been reformulated and commercialised. In most cases sodium salts were the replacement. However, to achieve the correct balance of gas release during baking, and optimum texture, flavour and color, combinations of NH_4HCO_3, $NaHCO_3$ and acidulant are often required (see below). Experiments have shown that NH_4HCO_3 can promote the production of AA in gingerbread [2]. NH_4HCO_3 increases the formation of sugar fragments (glyoxal and methylglyoxal) that react rapidly with Asn to furnish AA in higher yield than the native reducing sugars under "mild" conditions [3].

Coffee and Coffee Mixtures (e.g. roast and ground, soluble coffees, coffee mixtures)

Not applicable

Other considerations

Loss/change of color, loss of leavening/stack height, flavour defects, texture defects, increase in sodium.

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Recipe: Other minor ingredients

Potato (Potato products for frying, roasting, baking, but mainly French fries and potato crisps)

Lab & Pilot scale
Addition of competing amino acids such as glycine or deactivating Asn by calcium salts have shown reduction potential

Amino acids and calcium salts

Other amino acids may compete with Asn and can thereby reduce AA formation, or they may chemically react with AA for example through Michael addition. Shifting the balance away from Asn may help to reduce AA formation.

Potato snacks/cakes

- In a potato cake model, the combined treatment of citric acid and glycine (each 0.39% in the recipe) had an additive effect in reducing the AA concentration. Citric acid inhibits certain flavour formation which is compensated by the addition of glycine that favours the formation of certain volatiles [1].
- In pilot trials with potato-based snacks, at levels producing reduction, glycine addition also produced unacceptably high levels of browning and bitter off-flavours.

Industrial scale
Use of calcium can reduce AA in certain formulated products

- In certain potato-based pellet snacks ~1% addition of calcium chloride has given a ~20-80% reduction dependent on the product design. Too high levels of calcium salts can, however, generate off-flavours.

French fries

In lab trials glycine was not successful in lowering AA in French fries.

Lab & Pilot scale
The use of acids and their salts has proved promising at lab scale

Acidulants and vitamin C

The use of acidulants (acetic, citric acids) and ascorbic acid in the French fry industry proved to be promising at lab scale as a mitigation tool. However, great care must be taken in order to avoid sour off-taste.

Cereal (e.g. bread, crispbread, biscuits/bakery wares, breakfast cereals)

Lab & Pilot scale
Glycine addition changes product color/quality

Biscuits

Short sweet biscuits: glycine addition changes product color and furnishes products of unacceptable quality.

Gingerbread

In pilot trials, glycine addition (1 % in the recipe) decreased AA content ~2.5 fold and enhanced browning, but with a clear impact on the sensorial properties of the product [2].

Calcium gives variable results and most have adverse flavor effects

Trials at benchscale with added calcium salts gives variable results, most affecting product quality. None have been commercialised.

Industrial scale
Replacing fructose with glucose is very effective in reducing AA formation, particularly in recipes containing ammonium bicarbonate

Reducing sugars are responsible for many of the characteristic flavours and color in sweet biscuits. Fructose replacement by glucose retained original quality and texture in several commercial applications; paler colors were, however, acceptable. When glucose-fructose syrups are used, the fructose content should be as low as possible [3].

Industrial scale
For bread, addition of Ca^{2+} salts has shown to reduce the formation of AA

Bread

The fortification of flour with 0.3% calcium required by U.K. law for nutritional reasons gives a reduction in acrylamide of about 30%, and additional calcium fortification reduces the level still further (a similar effect can be obtained with magnesium addition). However, calcium propionate resulted in more than 90% increase in AA. This effect was not due to the propionic acid. Adding Ca^{2+} to bread via the tin releasing agent has a clear effect in reducing AA and may be an option as most of the AA is formed in the crust [4, 5].

Different studies show that glycine addition may lead to a reduction of AA in yeast-leavened bread, flat breads and bread crusts. However, it has also been suggested that addition of high amounts of glycine may lead to reduced yeast fermentation. Spraying glycine on the surface of bread dough (8-times consecutively) affords only a marginal reduction of AA (~ 16%) [6,7].

Lab & Pilot scale
Studies so far show that addition of Ca^{2+} has no significant effect on AA reduction

Lab & Pilot scale
Addition of glycine to breakfast cereals may be an option in some types of wheat flake, but is limited by impacting the product quality

Breakfast cereals

In breakfast cereal production, manufacturers in Europe generally use sucrose and small amounts of malt in the cereal itself because reducing sugars darken the cereal too much. Where alternatives to sucrose may be used, one should verify that these do not increase acrylamide levels. Honey, glucose, fructose and other reducing sugars are used in the sugar coat applied after toasting so they do not influence AA formation [5, 6]. Several trials show no effect of malt on AA formation, it seems likely that Asn content dominates at the levels of malt used.

Many breakfast cereals are fortified with Ca^{2+}, and manufacturers could explore the benefit for those not so fortified. The manufacturers who report trials have seen no significant effect of calcium addition. At pilot plant scale, glycine has been found to reduce AA formation by up to 50% in some types of wheat flake. The addition of glycine is limited by formation of dark color and a bitter taste.

Crispbread

The level of AA can be affected by glycine at 3% (w/w) resulting in a reduction by approx. 78%. However, the color may well be affected and an undesirable sweet flavour introduced [7]. Ca^{2+}, whilst only slightly reducing AA, has an adverse effect on the flavour and texture at pilot plant level.

Coffee and Coffee Mixtures (e.g. roast and ground, soluble coffees, coffee mixtures)

Lab scale: calcium and magnesium salts are not effective in reducing AA in coffee. Trials with chicory are ongoing.

Other considerations

- Potatoes:
  - environmental impact
  - calcium chloride: corrosion of the equipment
  - frying oil stability and organoleptic properties/ citric acid

- Bakery products for diabetics: greater potential of AA formation if sucrose is replaced by fructose.
- Calcium dosing and nutritional aspects to be considered.
- Potential adverse effect of glycine to be considered.

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Recipe: pH

Potato (Potato products for frying, roasting, baking, but mainly French fries and potato crisps)

Lab, Pilot & Industrial scale
Studies so far show that the effect of acids is dependent on the product design and in most cases leads to quality issues

Addition of citric or ascorbic acid has been found to successfully reduce AA and is used industrially for some types of formulated products. However, addition of acids to some potato-based snacks in pilot trials and French fries in lab scale tests produced strong off –flavours. This taste impact was not observed in other cases – potential for success is very variable dependent on product design. Addition of low levels of acids to raw materials has also shown synergistic benefits with calcium salts in small-scale trials for crisps. However the same tests for French fries at lab scale showed serious sour off-taste.

Cereal (e.g. bread, crispbread, biscuits/bakery wares, breakfast cereals)

Lab scale
Addition of organic acids has only been effective when combined with a change to leavening agents, and then with only marginal impact

Biscuits, Crispbread, Gingerbread

In the absence of ammonium raising agents pilot scale studies on biscuits have shown that pH and AA follow a linear trend with a reduction in AA of about 17% per unit drop in pH [1].

In laboratory experiments with an intermediate product (semi-sweet biscuit) a 20-30% reduction of AA was achieved by adding citric acid to reduce the pH [2].

Addition of citric and tartaric acid (~0.5% in the recipe) decreased AA content approx. 3-fold in gingerbread versus a control, but resulted in a product of insufficient quality (acidic taste, less browning) [3]. In crispbread and biscuits, the pH has an impact on the organoleptic properties of the final product.

Models have shown that in certain bakery products lower pH in combination with fermentation can lead to an increase in another undesired process chemical, namely 3-monochloropropanediol (3-MCPD) [4, 5].

Coffee and Coffee Mixtures (e.g. roast and ground, soluble coffees, coffee mixtures)

Not applicable

Other considerations

Organoleptic properties, product shelf-life and the potential formation of other undesired compounds such as 3-MCPD and 3-MCPD esters must be individually assessed. Citric acid is corrosive to some grades of steel and this should be considered before an extended factory trial is conducted.

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Recipe: Dilution

Potato (Potato products for frying, roasting, baking, but mainly French fries and potato crisps)

Industrial scale Partial replacement with ingredients lower in key reactants	For some preformed/reconstituted products, partial replacement of potato components by ingredients lower in key reactants reduces AA formation potential, and has been implemented, e.g. use of cereals with lower Asn amounts than potato (e.g. wheat, rice, maize) in the recipe [1].
Industrial scale Thicker strips can reduce AA through the surface area/volume effect	**Size dilution** AA is formed on the surface and the surface to volume ratio determines the quantity of AA expressed on the total product based on cooking to the same color endpoint. Hence decreasing the surface area to volume ratio, e.g. by thicker strips is one way of reducing AA.

Cereal (e.g. bread, crispbread, biscuits/bakery wares, breakfast cereals)

Industrial & Pilot scale Reducing wholemeal in the recipe affords lower AA in crispbread. However, impact on nutritional quality and organoleptic properties needs to be assessed	**Crispbread** If crispbreads are produced with cereal grains that are low in Asn, then consequently products low in AA are expected. It is possible to dilute the Asn-containing material in certain cases, and rye flour type 1800 replaced with type 997 was commercialised in one product. However, depending on the choice of the diluting material, this may change the product composition and characteristics considerably [2]. **Short sweet biscuits** The part replacement of wheat flour by rice flour is an effective measure.
Industrial scale Roasted nuts and dried fruits may contribute to the AA burden	**Breakfast cereals** All of the major grains may be used in breakfast cereals and some grains yield more AA than others within a common process. Wheat, barley and oats yield markedly more AA than maize, or rice. However, the choice of grain defines the food. Other ingredients used in cereal products may contribute to AA. Low-roast almonds contain about 10 fold less AA than high roast almonds. Peanuts and hazelnuts contain less than a fifth as much asparagine as almonds so they yield much less AA. Where baked pieces are used in muesli their recipe should be reviewed alongside the advice for biscuits. Some dried fruits (e.g. prunes, pears) were recently reported to contain AA [3]. Tests were therefore made of some ingredients commonly added to mueslis and flake-with-fruit cereals. Dried fruit and nuts may make up around 25-50% of muesli by weight, raisins and sultanas generally predominate. There was no measurable AA in raisins of several kinds and origins, dried apple, dried cranberries, candied papaya or candied pineapple. Low levels were found in dried bananas, dried coconut and prunes.

Baking a "larger product" can reduce AA through the surface area/volume effect	**Size dilution in bread and bakery products** AA is formed in the hot drying crust of bread and the crust (area) to crumb (volume) ratio determines the quantity of AA expressed on the total product. Hence decreasing the surface area to volume ratio, e.g. by producing a larger bread loaf, is one way of reducing AA [4].

Coffee and Coffee Mixtures (e.g. roast and ground, soluble coffees, coffee mixtures)

Industrial scale Recipe modification to accommodate lower %age of high AA-forming constituents	**Chicory** Lowering the chicory content by 3% in the recipes for coffee surrogates and partial substitution with, for example roasted barley, achieves marginal reduction but has an impact on organoleptic properties [1].

Other considerations

Cereals: Asn is more concentrated in the bran so use of refined grain may reduce AA. However, the fibre and other beneficial nutrients of bran would be lost, and the product characteristics would also change [5, 6].

Potatoes: implication for consumer acceptance and products characteristics.
Denomination of product may change based on the dilutions with other cereal grains.

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Recipe: Rework

Potato (Potato products for frying, roasting, baking, but mainly French fries and potato crisps)

Not applicable for French fries and standard crisps. However, re-work may have an impact in dough-based potato products, and needs to be considered.

Cereal (e.g. bread, crispbread, biscuits/bakery wares, breakfast cereals)

Pilot scale

There is evidence in the lab scale that elimination of rework provides a benefit in terms of AA reduction for some products. However, avoid where possible "dough aging" or rework of "aged" dough

Biscuits

Based on studies conducted in Germany, rework in certain bakery wares may have an impact on the amount of AA present in the final product [1]. For example, a 16% reduction of AA in gingerbread has been shown without rework.
In pilot studies with sweet biscuit dough it has been shown that more AA is formed in biscuits baked from older doughs (an increase of approximately 35% over 3 h). The extra AA could be accounted for by the measured increase in free Asn over time [2]. Hence best practice should avoid where possible "dough aging" or reworking of aged dough. However, the most recent survey shows that there is no evidence that elimination of rework provides any benefit in terms of AA reduction when applied on an industrial scale [3].
Other work conducted on non-fermented crispbread has shown no significant effect on the formation of AA in the product [4].

Breakfast cereals

For those breakfast cereals where rework can be used, no effect on the formation of AA is so far reported. The number of distinct processes and recipes is such that manufacturers should test each case.

Coffee and Coffee Mixtures (e.g. roast and ground, soluble coffees, coffee mixtures)

Not applicable.

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Processing: Fermentation

Potato (Potato products for frying, roasting, baking, but mainly French fries and potato crisps)

Lab scale
Lower levels of AA achieved by fermentation

Fermentation reduces levels of key reactants for the formation of AA, and lowers the pH.
Use of *Lactobacillus* to treat potatoes has been proposed. However, this option is currently not suitable for use in the context of present processes and available equipment.

Cereal (e.g. bread, crispbread, biscuits/bakery wares, breakfast cereals)

Lab, Pilot & Industrial scale
Lower levels of AA in fermented products. Extension of fermentation time in bread may be an option to lower AA levels

Use of lower gassing yeast to decompose Asn faster

In sweet biscuits, control of dough storage time may be a good practice

Biscuits
Some baked products, such as crispbreads and crackers, can be made from fermented doughs so as to develop specific textures and flavours. Compared to similar non-fermented products, the level of AA in the fermented variants is generally lower [1]. Yeast rapidly assimilates Asn and aspartic acid, as well as sugars. Crispbread, which is mainly produced with yeast, also shows significantly lower AA content for fermented variants versus cold bread (non-fermented variants). In crispbread manufacture, other factors such as biscuit thickness and baking conditions must be seen in perspective [2].
Biscuit and cracker doughs: long yeast fermentations are an effective way of reducing Asn levels. Fructose levels increase at moderate fermentation times, but the yeast later absorbed this, so the net effect on AA was beneficial [3]. However, no studies on increasing fermentation time in crackers were reported in the latest CAOBISCO survey [2].
The use of lower gassing yeast may be a mitigation option in some products since the latter is independent of Asn consumption. As more yeast activity is added this results in a faster decomposition of Asn at same overall gas generation rate [3].
Sweet biscuits: more AA is formed in doughs that have been allowed to age (35% increase over 3h), i.e. increase in free Asn in dough over time [3]. Hence, avoid adding "aged" dough. However, no studies on reducing dough hold time in hard sweet biscuits were reported in the latest CAOBISCO survey [2].

Lab scale
Extended yeast fermentation time may be an option but warrants further study

Bread
Lab scale trials have with flour salt and water doughs have shown that extended yeast fermentation may be one way to reduce AA content in bread [4]. Yeast preferentially removes Asn and a studies in the UK have shown a 50% reduction of AA after 1h fermentation time [3, 5]. However the reduction was less effective in commercial recipes that contained added improvers and bakery fat [3, 5]. These observations warrant further study.

Coffee and Coffee Mixtures (e.g. roast and ground, soluble coffees, coffee mixtures)

Not applicable [6].

Other considerations

Fermentation leads to increased glycerol and in combination with lower pH (addition of acids) may favour the formation of 3-MCPD [7].
Longer dough holding times and compatibility with industrial processing and effect on finished product quality need to be considered.

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Processing: Thermal input & moisture control

Potato (Potato products for frying, roasting, baking, but mainly French fries and potato crisps)

Industrial scale
Thermal input controls AA formation in the finished product

Crisps

Thermal input rather than temperature alone is critical to controlling product characteristics. This needs to take account of temperature and frying times and processing equipment.

- Different solutions to optimise thermal input to manage AA have been implemented in line with existing processing equipment.
- Vacuum frying offers an alternate thermal input control system, however this technology is not widely available and has limited throughput capacity [1].
- For manufacturers that use high temperature flash frying, rapid cooling helps to reduce AA formation.
- Moisture regime in the fried product is critical for successful industrial implementation of cooking control. Hence, it is important to fry to the maximum end moisture content that makes an acceptable product.

Industrial scale
For French fries, final preparation conditions are key

French fries

Par-frying does not produce significant levels of AA in the semi-finished product, nor does it determine the level in the final product.

Lab scale
Par-frying and high temp drying using dry steam

It is the final preparation that has the controlling influence.
See section “Final Preparation” for positive action taken.

Moisture content has a strong influence on the activation energy of browning and AA formation. At low moisture contents, the activation energy for AA formation is larger as compared to the one for browning. This explains why the end-phase of the frying process is critical and must be carefully controlled [2].

Cereal (e.g. bread, crispbread, biscuits/bakery wares, breakfast cereals)

The formation of AA during the baking of cereal products is closely related to the combination of moisture content and baking temperature/time (thermal input). If products could be baked to the same moisture, but with less color development, less AA would be formed even though longer baking times would be required [3, 4].

Industrial scale
Optimisation of thermal input has resulted in a reduction of AA in crispbread

Crispbread

In non-fermented crispbread, reduction in process temperature and oven speed reduced AA by approx. 75 %. The most important impact is coming from securing that the end humidity is as high as tolerable from a quality point of view. However, other products may suffer significant changes to color, flavour and texture [5 - 7].

Breakfast cereals

There is one report of a reduction in AA for a baked breakfast cereal when a two-zone oven with a cooler section replaced a single zone oven. Multi-zone ovens and toasters are the norm for large manufacturers and they have not found benefit without significant changes to organoleptic properties.

Two manufacturers have found that the conditions associated with

minimum energy use are also associated with least AA formation. Thus there may be a dual benefit from optimisation to minimise energy use in baking.
AA content is well correlated with moisture content however manufacturers generally apply both maximum and minimum ranges for moisture as a part of routine quality management.

Lab & Pilot scale

Alternative baking technologies such as infrared heating seem promising

Steam baking during the last 5 min. of bake is effective in reducing AA

Bread

In a UK study of bread produced by the Chorleywood bread process it was shown that AA formation could be reduced by taking some simple measures. These included avoiding excessive crust color generation, baking with lidded pans, and using falling oven temperature profiles [8].
The impact of new baking techniques such as air impingement and infrared radiation baking on AA formation in the crust has been studied within the Heatox project [1]. Using infrared heating, it was possible to reduce AA content in flat bread cakes by 60% with retained sensory properties. The effect of steam baking during the final part of baking was also studied and afforded a reduction of AA by 40% in white bread, maintaining the sensory quality [1, 9].

Coffee and Coffee Mixtures (e.g. roast and ground, soluble coffees, coffee mixtures)

Lab & Pilot scale

Roasting technologies beyond existing ones have been tested but do not indicate a mitigation opportunity

Coffee

At the beginning of roasting the AA formation starts rapidly. After reaching a maximum within the first half of the total roast cycle the AA level decreases with continued roasting. Final finished product levels are at only 20 - 30% of the maximum level, final concentration being dependent on the target degree of roast and the total roast time. Darker roasting in general, and extending the roast time by using lower roasting temperatures, tends to reduce the AA level but both parameters need to be fixed in narrow ranges to achieve the target flavour profile [5, 6, 10].

Different to most other food categories, the AA concentration in coffee decreases with increasing thermal input/darker roasting. The hypothesis is that at higher temperatures, as applied during coffee roasting, reactions leading to the depletion of AA dominate towards the end of the roasting cycle. These reactions are as yet not understood, but the hypothesis is supported by studies in model systems that show an increase and subsequent decrease of AA over temperature, explained by potential polymerisation or reaction of AA with food components [11].

Trials on new/alternative roasting technologies have been conducted. Using a steam/pressure roasting pilot plant unit resulted in a reduction potential of up to 10% in comparison with conventionally roasted sample of similar quality - not indicative of a significant mitigation opportunity.

Chicory

AA is formed at temperatures > 130°C, with a maximum at 145°C. Above 150°C, rapid decrease in AA due to process-related loss. Color development > 150°C due to caramelisation (degradation of sucrose). Decreasing the roasting temperature and concomitantly increasing the roasting time, favours the loss of AA.

Other considerations

Coffee: results have led to the conclusion that only very limited process options are available to reduce the AA level without affecting the quality respective the consumer acceptance of a product.

Crisps/French fries: effect of reducing the frying temperature on the fat content of the finished product (for example 9 mm fries: lowering the final cooking temperature from 180°C to 170°C leads to a 5 – 10 % increase in fat, when frying to the same color endpoint). Effect of incomplete cooking on moisture level in products and subsequent impact on product quality, shelf life, microbiological damage.

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Processing: Pre-treatment (e.g. washing, blanching, divalent cations)

Potato (Potato products for frying, roasting, baking, but mainly French fries and potato crisps)

Industrial scale
Blanching is the most important tool to control AA. Addition of sodium acid pyrophosphate reduces pH and thus lowers potential of AA formation

Lab & Pilot scale
For crisps, variable success with washing or pH control

French fries
The blanching process is the most important tool to control the reducing sugars (by removing and/or adding) to the required level of the color of the specification of the final product and thus AA [1].

Addition of sodium acid pyrophosphate directly after blanching of French fries is used to avoid discoloration of uncooked strips and has a secondary effect of reducing AA by lowering pH.

Crisps
Reducing sugars are often higher in the peel layer of some varieties especially for long-term stored potatoes, and peeling can help in overall reduction in these cases Blanching can lower reducing sugars in products depending on product thickness and where organoleptic qualities permit.

Different solutions to control key reactants, have been implemented, with different degrees of success, dependant on existing processing equipment:

- Washing
- PH control

Some AA reduction has been found in laboratory scale trials where potato slices have been soaked in solutions of various amino acids with differing impacts on the amount of AA reduction [2-5].

Calcium salts
The addition of di- and tri-valent cations has been proposed to reduce the formation of AA in several potato products.

- Laboratory research using calcium salts found AA reduction in potato crisps, not attributable to a lower pH [4]. Sensorial tests claimed good acceptability, but industry experience with calcium use suggests bitter off-flavours and brittle textures – this requires confirmation with products fried to same moisture content.
- The use of calcium salts (calcium lactate, calcium chloride) in the French fry industry proved to be promising at lab scale. However, care should be taken to avoid undesired textural effects (hard texture) and bitter off-taste. In addition, it should be noted that calcium is not compatible with pyrophosphate which is generally used to prevent grey discoloration.
- The use of magnesium chloride gave rise to serious off-tastes.

See also section "**Recipe: Other Minor Ingredients**" where the same tools can be envisaged as a pre-treatment for French fries and sliced potato crisps.

Cereal (e.g. bread, crispbread, biscuits/bakery wares, breakfast cereals)

Not applicable.

Coffee and Coffee Mixtures (e.g. roast and ground, soluble coffees, coffee mixtures)

Lab & Pilot scale Pre-drying of green beans and decaffeination have no significant impact on AA	**Coffee** Pre-drying of green beans: green coffee dried to lower moisture content prior to roasting (from a typical green coffee moisture of 10-12% to approximately 7%) did not show an impact of the initial green coffee moisture on the AA level in the roasted product [6]. Decaffeination: trials showed that roasting of decaffeinated green coffees (covering the commercially important decaffeination processes) results in AA levels of same magnitude as roasting of corresponding untreated coffees when roasted to comparable roasting conditions.

Other considerations

Not applicable

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Processing: Asparaginase

Potato (Potato products for frying, roasting, baking, but mainly French fries and potato crisps)

Lab scale Asparaginase may reduce AA in an optimised lab environment which, however, differs significantly from an industrial setting	**French fries** Asparaginase has been tested in a lab scale trial with promising results, provided that optimal temperature and pH conditions were assured for the enzyme activity. However, these conditions seem not compatible with realistic industrial processing conditions.
Lab & Pilot scale Asparaginase may reduce AA in reconstituted dough-based products but off-flavours can be created.	**Dough-based Potato Products** Asparaginase significantly reduces the levels of AA in potato-based dough products [1]. However, successful use with commercial, full-speed equipment has not yet been shown. The enzyme requires delicate balance of reaction conditions and contact time to be effective. In addition, the excess asparagine in potatoes may result in by-products (aspartic acid and ammonia) that can be formed in sufficient quantities to impart off-flavours.

Cereal (e.g. bread, crispbread, biscuits/bakery wares, breakfast cereals)

Industrial and Pilot scale Use of asparaginase is effective in biscuits, cereals, crisp bread, and is today applied to commercial products (e.g. gingerbread, crispbread, short sweet biscuits, RTE cereals, certain cereal-based snacks) with potential also in other biscuit and cereal product types	**Biscuits, gingerbread, and crispbread** Certain products are today produced with the use of asparaginase without any quality issues, e.g. gingerbread, crispbread and short sweet biscuits. More products are currently under evaluation and can be expected to be commercialised over the short to medium term. Asparaginase has a high potential for AA reduction especially in high moisture, neutral pH systems at elevated temperatures [2, 3]. **Hydrolysed Ready-to-Eat (RTE) Cereal Product** Industrial trials with a oat/rice/wheat cereal product that is roller dried showed that asparaginase - added during the wet mixing hydrolysis step - decreases AA by > 80 % in the final product. This approach is today applied to several commercial products. **Cereal-based snacks** Significant reductions (~70-90%) have been achieved through using asparaginase in certain cereal dough-based snacks, and are now in use at industrial scale. A minimum residence time dependent on asparagine levels is required to achieve maximal reduction.
Lab & Pilot scale No significant Asn reduction in a cooked and toasted coarse grain cereal	**Breakfast Cereals** Trials at laboratory and pilot scale in collaboration with an enzyme supplier confirmed that asparaginase was not effective for mitigation of AA. The breakfast cereal processes use a low moisture content which makes enzyme penetration into the grain or food matrix difficult. Further, many breakfast cereal processes use coarse flours or chopped grains which are not readily penetrated by the enzyme.

Coffee and Coffee Mixtures (e.g. roast and ground, soluble coffees, coffee mixtures)	
Lab scale Assessment of the opportunity to reduce the AA levels in roast coffee through a green coffee asparaginase treatment is under way	**Coffee** Preliminary results from lab/pilot plant studies show significant reductions in green coffee Asn levels after an enzyme treatment. This resulted in 20-40% lower acrylamide levels directly after roasting with magnitude depending on roast/blend conditions and when compared to an untreated coffee which has been roasted under same conditions. Although these preliminary findings are promising, the full impact assessment needs to include the following findings/considerations: • Sensorial evaluations of trial samples showed significant differences in flavour between untreated and enzyme treated roast coffees. • The trials have been done under lab/pilot plant conditions only. Scaling up will require significant modifications of the enzyme treatment conditions to determine if a viable, safe and sustainable commercial plant process is possible and practical. • The reported range of potential AA reductions is measured directly after roasting only. There are indications that this reduction will get almost "neutralized" during typical shelf life period of roast coffee. This is due to the known effect of AA decrease during storage/shelf life. **Chicory** Asn reduction: as described above.

Other considerations

The regulatory status and safety assessment *re* the use of asparaginase is now clearer within the EU. Denmark and France have approved the use of two commercial asparaginase enzymes in specific products.

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Final Preparation: Color endpoint

Potato (Potato products for frying, roasting, baking, but mainly French fries and potato crisps)

Industrial scale Cook to a golden yellow color. In-line elimination of dark crisps	**French fries** A study conducted by Ghent University* on the main parameters (reducing sugar content and color evaluation) linked to AA in the final product, revealed that the best correlation was achieved by color determination (Agtron process analyser) [1]. The cooking instructions on the packaging have been revised to achieve a golden yellow color for the finished product. Following these revised optimised cooking instructions results in lower AA levels [2,3] **Crisps** Elimination of dark colored crisps by in-line optical sorting has proven to be an effective measure to reduce AA.

Cereal (e.g. bread, crispbread, biscuits/bakery wares, breakfast cereals)

Industrial scale Color is a characteristic property of many products, but selected products could be modified without reducing consumer acceptability, e.g. if they are subsequently chocolate coated	The Maillard reaction, which leads to the production of AA, also produces the colors and flavours which give baked cereal products their essential characteristics. If, though, one was able to produce lighter colored and less baked products, but without increasing the moisture content, the AA level could theoretically be reduced [4, 5, 6]. Color endpoint is an approach applied for (i) hard sweet biscuits with a reported ca. 10 % reduction in AA and lighter color, and (ii) crispbread through reduced “final roast” with acceptable reduction in browning [6]. In some cases a darker color may be associated with less AA e.g. some breakfast cereals [4, 7]. In bread, the endpoint color does in most cases reflect AA content [8].

Coffee and Coffee Mixtures (e.g. roast and ground, soluble coffees, coffee mixtures)

Pilot & Industrial scale Color is an important process control point and linked to the sensory properties of the product	**Coffee** Color is an important indicator of roasting degree and directly related to the organoleptic properties of the product. Darker roast coffees have less AA than light roast coffees (see section “Processing: thermal input & moisture control” for more details) [4, 5, 9]. **Chicory** Color development results mainly from caramelisation of sugars, and color is an important end-point of roasting degree and attribute for consumer acceptance.

Other considerations

In the case of coffee, roasting to darker color is not considered an option to relatively lower AA due to the importance of the sensory attributes of the product. Additionally the effects of process changes on levels of desirable constituents (e.g. antioxidant capacity: studies have shown that in parallel to lower AA levels at darker roasting the antioxidant activity measured as *in vitro* radical scavenging capacity is decreasing as well [10]) and formation of other undesirable products under extreme roasting conditions need to be considered.

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Final Preparation: Texture/flavour

Potato (Potato products for frying, roasting, baking, but mainly French fries and potato crisps)

Not applicable	Modification of texture and flavour are not suitable as a tool to reduce AA, but are influenced by other interventions.

Cereal (e.g. bread, crispbread, biscuits/bakery wares, breakfast cereals)

Pilot scale Close correlation to moisture, an important organoleptic attribute	**Biscuits** It is unfortunate that the reaction leading to the formation of AA, the Maillard reaction is also that which develops flavour and color. In some products (e.g. gingerbread) reducing sugars, such as glucose or fructose, are deliberately added so as to achieve particular flavours (and color). Such products also tend to be higher in AA. Not to add the reducing sugars would reduce the amount of AA, but at the expense of flavour development. Products which are baked at a high temperature and to a low final moisture content, so as to have a 'crisp' texture, tend to be higher in AA. Those, such as shortbread, which are baked at low temperature and for a long time, are lower in AA. Individual studies warranted to assess feasibility and acceptance tolerance [1]. In crispbread, the thicker the bread, the lower the AA levels. This, however, significantly changes the product characteristics.

Coffee and Coffee Mixtures (e.g. roast and ground, soluble coffees, coffee mixtures)

Organoleptic properties are finely tuned by careful selection of green coffee blends, roasting conditions, and processing technologies	**Coffee** Flavour and aroma are crucial to the identity of the products, and any blend/ technology changes - however minor - to the existing products will have major impact on the organoleptic properties [2, 3, 4]. **Chicory** Valid as for coffee described above.

Other considerations

In the case of coffee, any minor changes to the blends / process will significantly impact the sensory properties and consumer acceptance.

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Final Preparation: Consumer Guidance

Potato (Potato products for frying, roasting, baking, but mainly French fries and potato crisps)

Advice to cooks and consumers French fries: Cook to a golden yellow color	**French fries** Follow exactly the product specific cooking instructions on the packaging. Frying products: • Cook at maximum 175°C for prescribed time • Do not overcook • Cook to a golden yellow color • When cooking small amounts, reduce the cooking time Oven products: • Do not overcook • Cook to a golden yellow color • When cooking small amounts, reduce the cooking time
Industrial scale "Fresh" prefabricates may have higher sugar contents toward end of product shelf life if blanching time is too short	A study performed in Switzerland has shown that "fresh" prefabricates of blanched French fries and hash browns stored at 4°C up to end of shelf life had relatively higher amounts of reducing sugars versus the same products that were kept frozen. The authors claim that residual enzyme activity (α-amylase) may slowly release reducing sugars during cold temperature storage [1]. The very short blanching time (5 min.) used in this study is not representative of industrial production of chilled or deep frozen French fries (blanching 15 - 45 min).

Cereal (e.g. bread, crispbread, biscuits/bakery wares, breakfast cereals)

Advice to cooks and consumers Toast bread to a light golden color	**Bread, toasted** Toasting bread to a light golden color.

Coffee and Coffee Mixtures (e.g. roast and ground, soluble coffees, coffee mixtures)

Typical brewing equipment transfers AA almost completely into the beverage. The cup/beverage concentrations for roast coffee and soluble coffee are similar	**Coffee** Brewing: typical household and non-household brewing equipment transfers the AA of the roast coffee almost completely into the beverage. Espresso brewing may however show lower transfer rates due to specific extraction conditions [2]. Soluble Coffee vs. Roast Coffee: similarly, in soluble coffee AA is efficiently extracted and concentrated into the final soluble coffee. After preparation/brewing the cup/beverage levels for roast coffee and soluble coffee are similar due to different typical recipes (with ca. 5-7g for roast coffee resp. ca. 2 g of soluble coffee per cup).

Other considerations

Other risk factors such as spoilage susceptibility resulting from mitigation measures must be taken into account.

Finish frying (catering)/ optimization of the fryer temperature program is important to ensure that temperature does not drop below a given limit after loading the fryer [3].

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Annex 1 Tools successfully tested at industrial level to mitigate acrylamide (AA)

	Toolbox Compartment			
	Agronomical	Recipe	Processing	Final Preparation
French Fries	• Choose potato varieties with low reducing sugar levels. • Store potatoes at > 6°C • Control the levels of reducing sugar´ or fry test the potatoes		• Blanch potato strips in hot water to remove reducing sugars • Cut thicker strips	• Follow on-pack instructions • Control the temperature & time of final cooking • When cooking smaller amounts, reduce cooking time • When frying do not cook >175°C • Aim for light golden color
Potato crisps	• Choose potato varieties with low reducing sugar levels. • Lot selection of potatoes suitability for the product type should be based on reducing sugars content or color assessment of a fried sample. • Long-term storage of potatoes should be > 6°C.	• Use of calcium salts can reduce AA in formulated potato-based products • Use of acids shown to reduce AA in some types of formulated products.	• Control the temperature/time cooking profile and final moisture control • In flash frying: ensure rapid cooling • In-line color sorting to remove dark crisps	• Aim for a light golden color
Bread	• For wheat grain, the importance of maintaining sulphur levels must be stressed to farmers	• Avoid adding reducing sugars in the recipe • The addition of calcium salts e.g. calcium carbonate and sulphate may reduce the formation of AA	• Control the baking time and temperature to prevent excessive browning in the crust	• When toasting aim for a light golden color
Crisp bread	• For wheat grain, the importance of maintaining sulphur levels must be stressed to farmers		• Non-fermented crisp bread control process temperature and oven speed • Control the final moisture content • Asparaginase is a tool for non-fermented crips bread	
Biscuits/bakery wares	• For wheat grain, the importance of maintaining sulphur levels must be stressed to farmers	• Replacement of ammonium bicarbonate with other raising agents • If possible avoid using fructose	• Asparaginase is a tool for certain biscuit and cereal applications • Do not over bake	
Breakfast cereals	• For wheat grain, the importance of maintaining sulphur levels must be stressed to farmers	• Minimise reducing sugars in the cook phase • Consider the introduction of other inclusions e.g. roasted nuts, tried fruits	• Do not over bake or over toast • Manage the toasting to achieve an uniform color for the product	