FoodDrinkEurope
Acrylamide Toolbox 2013

Source: FoodDrinkEurope – 10 January 2014
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Key Changes to the Acrylamide Toolbox since 2011

- Adjusted the categorisation of the tools to identify:
  (1) Tools that are proven at industrial scale (**Commercial applications**)
  (2) Emerging tools that may have had some success but not shown consistent results **(Development**)
  (3) Technologies tested at bench-scale that have only been tested in a laboratory environment or have some impact on product attributes (**Research**).
  This update is intended to help regulators and other users understand what they can expect to see implemented in the various food categories.
- Removed those food categories for which there is no activity .
- Examined existing tools and adjusted the categories as approporiate.
- Potato-based products have been split into two categories – Potato Based Snacks versus French Fries and other cut, (deepfried) Potato Products. Whilst certain tools are applicable to both categories there are a number of tools that when implemented in both categories may fail to deliver the same degree ofreduction in acrylamide due to processing and finished product attributes. French fries and other cut, (deep-fried) Potato Products are not ready-to-eat products such as the Potato Based Snacks, but still need final cooking by food business operators or final consumers at home.
- Latest scientific publications and project updates.

Summary

The FoodDrinkEurope acrylamide "Toolbox" reflects the results of >10 years of cooperation between the food industry and national authorities of the European Union to investigate pathways of formation of AA and potential intervention steps to reduce exposure.
The aim of the Toolbox is to provide national and local authorities, manufacturers (including small and medium size enterprises, SMEs) and other relevant bodies, with brief descriptions of intervention steps which may prevent and reduce formation of acrylamide in specific manufacturing processes and products. It is in particular intended to assist individual manufacturers, including SMEs 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 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.
Previous versions of the Toolbox were structured around 14 different parameters ('Tools'), grouped together in four main categories ('Toolbox compartments') that could be used selectively by food producers in order to lower acrylamide levels in their products. The four compartments refer to (i) agronomical factors, (ii) food recipe, (iii) processing and (iv) final preparation.
During the latest revision of the Toolbox, it became clear that this format no longer represents the most practical way of presenting tools for individual products types. The format also made it difficult for the individual sectors to update the parameters.

Taking into account the publication of the *CODEX CODE OF PRACTICE FOR THE REDUCTION OF ACRYLAMIDE IN FOODS (CAC/RCP 67-2009)*, this latest revision of the Toolbox has therefore been restructured around the three main product categories with higher risk of acrylamide formation, namely: potatoes, cereals, and coffee. These are then sub-divided into compartments and the individual tools.

This revised structure increases the overall length of the Toolbox, but allows the reader to better comprehend the parameters which may be applied selectively 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 guide. 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: FoodDrinkEurope

As of the 12th Revision of this document in 2009, FoodDrinkEurope has sought to include information from food and beverage manufacturers in the USA, provided through the Grocery Manufacturers Association (GMA). This corroborates the global applicability and use of the acrylamide Toolbox.
Lastly, to assist SMEs in the implementation of the Toolbox, FoodDrinkEurope 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 22 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. The latest JECFA evaluation of acrylamide published in 2010 confirmed the previous evaluations and concluded that a human health concern is indicated. However, JECFA also concluded that more data is needed to better estimate the risk from food consumption. At the EU level, progress in the research on acrylamide has been shared openly and regularly through stakeholder meetings, workshops and forums. The present text is the 14th edition incorporating latest developments and knowledge.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 such as 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 FoodDrinkEurope, to identify and accelerate the

implementation of possible steps to reduce acrylamide levels in foods. These efforts are also intended to explore how the learning's developed by industry might also be applied in home cooking and catering which contribute to more than half of the dietary intake of acrylamide.
Efforts of food industry are on-going, as in many cases there are no easy (single) solutions due to the complexity of factors to be considered. This requires further research, which also includes work with academics e.g. to reduce the natural occurrence of the precursor (e.g. asparagine) in raw materials.

Acrylamide Formation

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.

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. 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 colour, 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.
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, colour 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.
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 highly dependent on natural variations in raw materials.
Whilst the acrylamide Toolbox can provide useful leads, its practical application in domestic cooking, and catering requires additional work.

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 each product category, have been identified. (note - each parameter may not be applicable within all categories of products).

Agronomy

- Reducing Sugars
- Asparagine

Recipe (Basic Formula, Ingredients, Product Form)

- Raising/Leavening Agents
- Other Minor Ingredients (e.g. glycine and divalent cations)
- pH
- Rework
- Dilution/Piece Size
- Fermentation

Processing

- Thermal Input & Moisture
- Asparaginase
- Pre-Treatment
- Finished Product Colour
- Texture/Flavour

Final Preparation

- Consumer Guidance

The main food categories / sub-categories defined in the Toolbox are as follows:

Potato-based products

- Snacks made from potatoes (sliced) or fabricated products (made from potato based ingredients e.g. flakes, granules, French Fries and other cut, (deepfried) Potato Products

Cereal/Grain based products

- Bread
- Crisp bread
- Breakfast cereals
- Biscuits / bakery wares [classification as defined in the CAOBISCO study 2008, including crackers, semisweet products, pastries, short sweet biscuits, wafers, cakes and gingerbread]

Coffee, roasted grain and substitutes

- Roast and ground coffee
- Instant (soluble) coffee
- Coffee substitutes

Infant Foods

- Baby biscuits
- Infant cereals
- Baby foods other than cereal based foods

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 and desired final product design.
The summaries in this document also specify the level of experience available for a proposed intervention, i.e. (i) experimental work usually done at the laboratory/bench, (ii) development, representing trials evaluated mainly at the pilot scale or (iii) commercial applications, as outlined below.

- **Commercial Application:** 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 and desired final product design. The validation of the suggested tools was assessed over the product shelf life. The legal status of the proposed measures has been evaluated
- **Development:** These concepts have been evaluated in the pilot plant or in test runs in the factory and were successful to some extent to deliver measurable reductions, but not yet applied successfully under commercial production conditions. These tools may still have some risks to product attributes and/or inconsistent mitigation results. New applications of existing technologies. Tools at agronomical level.

- **Research:** 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.

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 FoodDrinkEurope.
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 FoodDrinkEurope's intention to continuously update the acrylamide Toolbox so as to reflect such developments.

The Concept of ALARA

"ALARA" is an acronym for the concept "As Low As Reasonably Achievable".
ALARA means that a Food Business Operator (FBO) should take every reasonable measure to reduce the presence of a given contaminant in a final product taking into account other legitimate considerations.
To ensure continuing compliance with the ALARA concept, the FBO should monitor the effectiveness of the implemented measures and should review them as necessary.

ALARA as Applied to Acrylamide

In the context of acrylamide and other process contaminants, which are the result of naturally occurring chemical reactions in heated foods and for which there are currently no levels that regulators have agreed upon as being 'safe', ALARA means that the FBO should make every reasonable effort (based upon current knowledge) to reduce levels in final product and thereby reduce consumers exposure. ALARA could for example mean that an FBO changes parts of a process or even a whole process if technologically feasible.

The tools identified within the FoodDrinkEurope Toolbox are potential measures designed to limit acrylamide levels in final product through interventions at various stages of production (agronomical, recipe, processing, and final preparation). They are based upon scientific knowledge and practical application in specific circumstances.

As technology develops, new and better tools for acrylamide reduction may become available. As part of a continuous process FBOs should review what tools are available on regular basis, and consider whether they can implement these tools into their processes or their product.

If a FBO chooses not to implement an available tool then the onus should be placed upon that same FBO to demonstrate why its application is believed to be unreasonable or ineffective.

Considerations may include:

- potential impact that use of a known acrylamide mitigation tool will have upon levels of acrylamide in the final product
- potential impact that use of a known acrylamide mitigation tool may have upon the formation of other process contaminants (e.g. furan) and/or reduction in control of other hazards e.g. microbiological

- feasibility of implementing the identified controls, e.g. legal compliance, commercial availability of the mitigation tool, occupational health hazards, timescales and costs associated with upgrading or replacing plant equipment.
- impact that use of a known acrylamide mitigation tool will have on organoleptic properties and other quality aspects of the final product as well as product safety (the ideal method would have no adverse effects)
- known nutritional benefits of using certain ingredients in preference to others, e.g. use of whole grain cereals instead of refined cereals.

Methods of Analysis and Sampling

Today, many laboratories offer sensitive and reliable methods to analyse acrylamide in a wide range of foods. Previous issues with the extractability of acrylamide in certain food matrices were raised showing that a high extraction pH may significantly enhance the yield of acrylamide versus extraction under neutral pH. Work done by independent research groups 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 of reliable analytical methods is of major importance. Moreover, an area that requires more attention in the analytical arena is the development of rapid test methods for acrylamide that are cheap and reliable, particularly methods that can be used by non-technical personnel in a manufacturing environment.
The main challenge for the analyst is the high variability of the products. This starts from the natural variability of a given raw material, for example 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, processing equipment capability, 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.

In September 2010 the CEN received confirmation that the European Commission had approved Mandate M/463 on METHODS OF ANALYSIS FOR FOOD CONTAMINANTS (process contaminants).

M/463, which has been allocated to the committee *CEN/TC 175/WG 13 'Process Contaminants'*, is for the delivery of a total of nine analytical standards, two of which are for AA. These standards are:

- determination of acrylamide in potato-based products, cereal based products and coffee with LC-MS (Deadline for requested deliverable is 31 December 2013)

- determination of acrylamide in potato-based products, cereal based products and coffee with GC-MS (Deadline for requested deliverable is 31 December 2016).

It is hoped that the standardisation of methods through CEN will, in the longer term, lead to more accurate assessments of acrylamide levels in the final product.

It is best practice to check accreditation (ISO/IEC 17025:2005) and validated methods of analysis before choosing to work with a laboratory.

Measurement Uncertainty

Whilst many laboratories are able to analyse for acrylamide, there are many issues regarding intra-laboratory and intra-country competency that should be considered. For example in its 2009 scientific report on the *Results on the monitoring of acrylamide levels in food* (EFSA Scientific Report (2009) 285: 11*),* EFSA identified that for some commonly employed analytical methods there were still significant measurement uncertainty (MU) between results presented by different European Union Member States. The reported MU for LC-MS ranged between 6 and 53%, and for GC-MS between 0 and 64%. Furthermore the minimum and maximum values for LOD and LOQ ranged from 1 to 250 and from 3 to 500 µg/kg, respectively.

Therefore a further potential confounding factor for FBOs, on top of the natural variability within product, will be laboratory to laboratory and country to country comparability.

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-license the rights to food manufacturing and processing companies to incorporate asparaginase in their food production processes to lower the amounts of acrylamide.

GRAS status ("Generally Recognized As Safe") has been obtained from the USA 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.

Asparaginase - under specific conditions of use - is approved in the countries listed below (non exhaustive list based on information provided by food manufacturers). However, note that this list covers countries where the products are either officially approved or where there are no legal restrictions for marketing.:
Angola, Armenia, Australia, Austria, Azerbaijan, Bahrain, Bangladesh, Barbados, Belgium, Benin, Bolivia, Bosnia-Herzegovina, Botswana, Burkina Faso, Burundi, Canada,Cambodia, Cameroon, Central African Republic, Chile, China, Columbia, Congo, Croatia, Cuba, Cyprus, Czech Republic, Denmark, Ecuador, Equatorial Guinea, Estonia, Ethiopia, Faroe Islands, Finland, France, Gabon, Georgia, Germany, Ghana, Great Britain, Greece, Guinea, Guinea-Bissau, Hong Kong, Hungary, Iceland, India, Indonesia, Ireland, Italy, Ivory Coast, Jordan, Kenya, Laos, Latvia, Lebanon, Lesotho, Liberia, Libya, Lithuania, Luxembourg, Macedonia, Madagascar, Malawi, Malta, Mexico, Mozambique, Myanmar, Namibia, Nepal, Netherlands, New Zealand, Nigeria, Norway, Oman, Pakistan,

Paraguay, Poland, Portugal, Qatar, Romania, Ruanda, Russian Federation, San Marino, Senegal, Singapore, Slovenia, Somalia, South Africa, Spain, Sri Lanka, Swaziland, Sweden, Syria, Tanzania, Togo, Tunisia, Uganda, United Arab Emirates, USA, Vanuatu, Venezuela, Yemen, Zambia, Zimbabwe.

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. Additional considerations are as follows:

- 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 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.

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

It is important that food manufacturers 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 should be noted that measures aimed at reducing levels of acrylamide cannot be isolated from other considerations. Precautions need to be taken to avoid compromising the existing chemical and microbiological safety of the food. The nutritional qualities of products also need to remain unimpaired, together with their organoleptic properties and associated consumer acceptability. This means all minimisation strategies need to be assessed with regards to their benefits and any possible adverse effects. For example:

- When preventative measures for acrylamide are considered, checks should be made to ensure that they will not result in an increase in other process contaminants. These include N-nitrosamines, polycyclic aromatic hydrocarbons, chloropropanols, ethyl carbamate, furan, heterocyclic aromatic amines and amino acid pyrolysates.
- Preventative measures devised for acrylamide must not compromise the microbiological stability and safety of the final product. In particular, attention needs to be paid to the moisture content of the final product, and in the case of jarred baby foods that the heat treatment is effective to reduce the microbial load to an acceptable level.
- It is recognised that acrylamide mitigation can cause detrimental changes to the organoleptic properties of the final product. The formation of acrylamide is intimately associated with the generation of the characteristic colour, flavour and aroma of cooked foods. Proposed changes to cooking conditions, or

indeed raw materials and other ingredients, must be assessed from the perspective of the acceptability of the final product to the consumer.

- Preventative measures devised for acrylamide must not compromise the nutritional quality of the product as outlined above.
- Formal safety assessments, efficacy-in-use demonstration and regulatory approval may be needed for potential new additives, enzymes, and processing aids such as asparaginase. Some companies are producing asparaginase for use in food products and some countries have approved it as a processing aid.
- It should be noted that the extent of acrylamide formation can be quite variable e.g. within a production batch made at the same manufacturing plant, or between plants using the same process, ingredients and formulations.
- Manufacturers need to be aware that variability in incoming raw materials and poorly controlled heating devices can complicate trials of mitigation strategies, by potentially obscuring changes in acrylamide levels.

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. 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

ALARA: As Low As Reasonably Achievable

Asn: Asparagine

BBSRC: Biotechnology and Biological Sciences Research Council (UK)

BLL: Bund für Lebensmittelrecht und Lebensmittelkunde e.V. (German food and drink manufacturers' association)

CAOBISCO: Association of the Chocolate, Biscuits and Confectionery Industries of the EU, For training and other sector specific activities please consult the following website: http://caobisco.eu/

CEEREAL: European Breakfast Cereal Association, For training and other sector specific activities please consult the following website: http://www.ceereal.eu/asp2/welcome.asp

FOODDRINKEUROPE: Confederation of the Food and Drink Industries of the EU

CCCF: Codex Committee on Contaminants in Foods

CEN: European Committee for Standardization/Comité Européen de Normalisation

Codex: Codex Alimentarius Commission

EC: European Commission

ECF: European Coffee Federation, For training and other sector specific activities please consult the following website: http://www.ecf-coffee.org/

ESA: European Snack Association, For training and other sector specific activities please consult the following website: http://www.esa.org.uk/**EFSA**: European Food Safety Authority

EUPPA: European Potato Processors' Association, For training and other sector specific activities please consult the following website: http://www.euppa.eu/en/

FBO: Food Business Operator

FDA: Food and Drug Administration

FEI: Research Association of the German Food Industry

GAP: Good Agricultural Practice

GC-MS: Gas Chromatography-Mass Spectrometry

Gln: Glutamine

GMA: Grocery Manufacturers Association

GRAS: Generally Regarded as Safe

ISO: International Organization for Standardization

JECFA: Joint FAO/WHO Committee on Food Additives

LC-MS: Liquid Chromatography-Mass Spectrometry

LINK: UK government/industry co-funded collaborative research for innovative and industrially-relevant research

LOD: Limit of Determination

LOQ: Limit of Quantification

MU: Measurement Uncertainty

SCF: Scientific Committee on Food (EU)

SME: Small and Medium size Enterprises

WHO: World Health Organization

Potato Based Snacks

Snacks made from potatoes whether sliced and fry or fabricated products made from potato based ingredients (flakes, granules, etc.)

1. Agronomy: Reducing Sugars

General Considerations

Reducing sugars are one of the key reactants for the formation of AA. The sugar content of the tuber correlates well with the AA concentration in the product especially if the fructose/asparagine (Asn) ratio is < 2.The concentration of reducing sugars is generally regarded as a good indicator of the relative AA forming potential of different batches of tubers of the same potato variety.

Commercial Application	• Minimising reducing sugars is part of standard manufacturing practices • Control of tuber storage temperature identified as good practice • Use of sprout suppressants to prevent sweetening during storage • These measures are implemented throughout the industry	Controlling reducing sugar is currently the primary measure employed by the industry to reduce AA levels in potato based snacks. This is achieved through: • Selection of potato varieties with low reducing sugars that are suitable for the product type. • Minimising the risk of high reducing sugars by growing those low sugar varieties best suited to the local growing conditions, by appropriate field selection, and by adherence to agronomy best practice. • Ensuring tubers are mature at time of harvesting (immature tubers tend to have higher reducing sugar levels). • Selecting lots based on reducing sugars content (crisp industry) or colour assessment of a fried sample (crisp industry/French fry industry) - good correlation between reducing sugars content and colour. • Controlling storage conditions from farm to factory (e.g. temp. >6°C identified as good practice for long term storage, use of sprout suppressants such as chlorpropham (CIPC) in accordance with legislation and GAP, reconditioning at higher temperature. (e.g. ambient) over a period of a few weeks). • Store potatoes only for the period of time recommended for each specific variety. • Selecting potato based raw materials (flakes or granules) with the lowest possible reducing sugar level that delivers the appropriate product attributes.

Future Opportunities

Breeding new potato varieties with lower reducing sugar content and/or less cold sweetening effect.Further optimise agricultural practices to minimise reducing 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 nitrogen fertilisation.

2. Agronomy: Asparagine

Development	Selection of crop varieties on the basis of typical free Asn: total free amino acids ratio	Recent research suggests that the impact of farming practices (e.g. fertiliser regimes) may have an effect on amino acid ratios in potatoes. Sulphur deprivation may alter the ratio of free Asn: total free amino acids in a tuber and research have suggested that this ratio is potentially of greater significance to the formation of AA in potatoes than previously thought: in particular in the Asn: Gln ratio. So far no optimum amino acid ratio in potato has been established.
Research	Control of Asn levels in tubers Farming practices, e.g. fertiliser regimes Laboratory and field studies with new potato varieties	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 reducing sugar levels. On its own, the reducing sugar concentration of potatoes is not always directly proportional to the acrylamide concentration observed in a fried potato product. The concentration of free Asn and the ratio of free Asn to other free amino acids (of which glutamine is by far the most abundant) should also be considered and may be better indicators of the relative acrylamide risk of different potato varieties. 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 (eg fertiliser regimes) on Asn/amino acids levels In potatoes, the effect of sulphur is uncertain, and any advice on S-fertilization to farmers would be premature based on the studies conducted so far. An increased ratio of other amino acids to Asn results in competition for reactants during the Maillard reaction, potentially affecting the proportion of AA formed in the overall Maillard process. New potato varieties with silenced Asn synthase genes in the tuber have up to 20-fold reduced amounts of free Asn. Heat processed derived from such tubers show comparable sensorial properties to their conventional counterparts, and expectedly much lower levels of AA. This work is based on laboratory trials. In field trials in the U.S., new potato varieties with silenced Asn synthase genes in the tuber have up to 5-fold reduced amounts of free Asn. Heat-processed products derived from such tubers show comparable sensorial properties to their conventional counterparts, and expectedly much lower levels of AA (50-75% reduction). As of writing such potatoes are not yet approved for commercial production in the EU or US, therefore the regulatory status should be checked.

3. Recipe: Other Minor Ingredients (amino acids, calcium salts and co-ingredients)

<table>
<tr><td>Commercial Application</td><td>Certain minor or co-ingredients have the potential to contain comparatively high levels of AA which could impact upon levels in the final product</td><td>Fabricated potato based products

Co-ingredients
Techniques associated with the production of some minor ingredients could result in high levels of AA in those ingredients, e.g. pre-processed cereals, processed sugars such as molasses, or certain processed spices/flavourings. These could potentially raise overall AA levels in the final product.

There may be an impact with co-ingredients included into a composite product (e.g. pre-processed cereal pieces, vegetables, nuts, seeds) where they could be “cooked” several times over. This needs to be deliberately taken into account in product design, processing practices, and issues with processing equipment and performance.</td></tr>
<tr><td>Development</td><td>Amino acid and calcium salt addition may impact important product attributes</td><td>Fabricated potato based products

Amino acids
• In pilot trials with potato-based snacks, at levels showing reduction, the addition of amino acids can produce unacceptably high levels of browning and bitter off-flavours.
• At lower levels, amino acid use may have an AA-reducing effect while still acceptable colour impact.
• 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.

Calcium salts
• Treatment of potato flakes with calcium salts during their production have demonstrated 30-40% reduction dependent on the product design and formulation. Too high levels can, however, generate undesirable product attributes.</td></tr>
</table>

4. Recipe: pH

Commercial Application	The use of acids and their salts has been proven effective at industrial scale in some products	**Fabricated potato based products** **Addition of Acids** Addition of citric or ascorbic acid has been found to successfully reduce AA and is used industrially for some types of fabricated potato based products. However, addition of acids to some products produced strong off –flavours. This taste impact was not observed in other cases – potential for success is very variable and dependent on product design. Studies so far show that the effect of acids is dependent on the product design and can lead to quality issues unless carefully controlled.
Research	Combined treatments of acid and glycine can be applied to balance flavour formation	**Acidulants in combination with glycine** 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.

5. Recipe: Dilution and Piece Size

Commercial Application	Partial replacement with ingredients lower in key reactants can be effective	For some pre-formed/reconstituted products/ fabricated potato based products, partial replacement of potato components by ingredients lower in key reactants reduces AA formation potential, e.g. use of cereals with lower Asn amounts than potato (e.g. wheat, rice, maize) in the recipe.
	Slice/piece thickness can reduce AA through the surface area/volume effect when taking into account finished product moisture and fry temperature profile	In products that are fried to low moistures, reducing the surface to volume ratio (by producing a thicker cut crisp) can result in increased AA as it will require a higher thermal input for the same fry time or longer fry time at the same thermal input to reach the same moisture end. A thin cut potato crisp product would require less thermal input for the same fry time to reach the same moisture endpoint, so in practice would form less AA.

6. Recipe: Fermentation

Research	Lower levels of AA can be achieved by fermentation	**Fabricated Potato Based Products** Fermentation reduces levels of key reactants for the formation of AA, and lowers the pH. The 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.

7. Processing: Asparaginase

Commercial Application	Asparaginase may reduce AA in reconstituted dough-based products but off-flavours can be created in some recipes	**Fabricated Potato based Products** Asparaginase significantly reduces the levels of AA in fabricated potato-based products. On a commerical scale the enzyme's effectiveness is recipe and process dependent and requires a delicate balance of reaction conditions and contact time to be effective. In some recipes, the excess Asn in potatoes may result in by-products (aspartic acid and ammonia) that can be formed in sufficient quantities to impart off-flavours.
Research	Asparaginase may reduce AA in an optimised lab environment which, however, differs significantly from an industrial setting	**Potato crisps** Treatment of fresh slices of potato with asparaginase has been found to be ineffective in pilot and industrial settings. This is because the enzyme cannot penetrate the potato slice/cell walls sufficiently to act upon the Asn. Extended soak/pre-treatment of such thin slices of potato with asparaginase results in disintegration of the slice structure.

8. Processing: Thermal Input & Moisture

General Considerations

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 at a lower product temperature to optimise colour development and minimize AA formation.
For French fries the finished frying / (oven) cooking of the prefried potato product is done by the professional end-user or by the consumer at home (see guidelines under 'final preparation').

Commercial Application		
	Thermal input controls AA formation in the finished product Controlling moisture helps to manage cooking control For French fries, final preparation conditions are key	**Crisps and fabricated potato based products** 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. Additionally, vacuum frying may not deliver desired product attributes given the lack of maillard compounds formed. • 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.

Other Considerations

Effect of reducing the frying temperature on the fat content of the finished product (for dropping the outlet frying temperature by about 5ºC from design will lead to an increase in fat, when frying to the same colour endpoint). Effect of incomplete cooking on moisture level in products may have a subsequent impact on product quality, shelf life, and/or microbiological damage.

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

Commercial Application	Blanching of potato slices/pieces prior to processing	Potato Sticks – blanching of potato sticks type products sliced from whole potatoes to remove sugars has been proven to reduce AA levels without the negative affects of flavour, texture and oil content update as see with potato crisps.
Development	Peeling and washing of potatoes prior to processing Blanching of potato slabs prior to flake/granule production and blanching of potato slices The addition of di- and tri-valent cations has been proposed to reduce the formation of AA	**Crisps and fabricated Potato Based Products** 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. However, impact of peeling on overall AA levels is highly dependent on the potato variety and season. Blanching may be an option in the production of potato flour, flakes or granules. Blanching of sliced potato crisps is not desired as it results in loss of flavour, loss of texture and increased oil content due to the disruption to the potato cells on the surface of the slices, and is thus not a preferred mitigation tool. **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. Sensorial tests from laboratory scale 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 laboratory 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 disodium diphosphates which is generally used to prevent grey discoloration. • The use of magnesium chloride gave rise to serious off-tastes.

Research	For crisps, variable success with washing or pH control	Different solutions to control key reactants, have been implemented, with different degrees of success, dependant on existing processing equipment: • Slice 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.

10. Processing: Finished Product Colour

Commercial Application	In-line optical sorting can be an effective measure to remove dark products Finished product colour	**Crisps and fabricated potato products** Elimination of dark coloured crisps/ products by in-line optical sorting has proven to be an effective measure to reduce AA. Dark coloured chips come from individual potatoes that are very high in reducing sugars and can increase the AA level of a given sample by 25-50% depending on the amount of dark coloured crisps/products in the sample Removal of 'fines' from frying oil should be part of good manufacturing practices to avoid any dark over-cooked pieces presence in pack There is evidence to suggest that consumers dislike very pale potato crisps. Lighter coloured potato crisps could be an acceptable product for a consumer, if other organoleptic properties such as taste and texture are managed. Use of ingredients such as paprika to add back color post frying may compensate for lighter coloured potato crisps by providing additional colour.
Research	On-line or Near-line colour measurement	Continuous measurement of finished product colour can (if properly calibrated) be a reliable predictor of finished product AA-levels. There are many watch-outs with the practical implementation and use of colour as a tool for measuring AA, not least because AA is itself colourless. Inference of AA based on colour would be a proxy or by product of Maillard chemistry ie colour formation which may not be equally well correlated for all product types

Other Considerations

As part of standard quality procedures, manufacturers should implement controls which may have an indirect impact upon AA levels in the final product e.g. management of oil quality to ensure cooking temperatures are optimally maintained.
Modification of texture and flavour are not directly suitable as a tool to reduce AA, but are impacted by other interventions.

French Fries and other cut, (deepfried) Potato Products

1. Agronomy: Reducing Sugars

General Considerations

Reducing sugars are one of the key reactants for the formation of AA. The sugar content of the tuber correlates well with the AA concentration in the product especially if the fructose/Asn ratio is <2.
The concentration of reducing sugars is generally regarded as a good indicator of the relative AA-forming potential of different batches of tubers of the same potato variety.

Commercial Application	Minimising reducing sugars is part of standard manufacturing practices Control of tuber storage temperature identified as good practice Use sprout suppressants to prevent sweetening during storage These measures are implemented throughout the industry	Controlling reducing sugar is currently the primary measure employed by the industry to reduce AA levels in French fries. This is achieved through: • Selection of potato varieties with low reducing sugars that are suitable for the product type.Minimising the risk of high reducing sugars by growing those low sugar varieties best suited to the local growing conditions, by appropriate field selection, and by adherence to agronomy best practice. • Ensuring tubers are mature at time of harvesting (immature tubers tend to have higher reducing sugar levels). • Selecting lots based on colour assessment of a fried sample,as there is a good correlation between reducing sugar content and final fried colour. • Controlling storage conditions from farm to factory (e.g. temp. 6-8°C identified as good practice for long term storage, use of sprout suppressants such as chlorpropham (CIPC) in accordance with the law and with GAP, reconditioning at higher temperature. (e.g. ambient) over a period of a few weeks before delivery). • Store potatoes only for the period of time recommend for each specific variety.

Other considerations

Breeding new potato varieties with lower reducing sugar content and/or less cold sweetening effect.Further optimise agricultural practices to minimise reducing 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 nitrogen fertilisation.

2. Agronomy: Asparagine (Asn)

Development	Selection of crop varieties on the basis of typical free Asn: total free amino acids ratio	Recent research suggests that the impact of farming practices (e.g. fertiliser regimes) may have an effect on amino acid ratios in potatoes. Sulphur deprivation may alter the ratio of free Asn: total free amino acids in a tuber and research have suggested that this ratio is potentially of greater significance to the formation of AA in potatoes than previously thought: in particular in the Asn: Gln ratio. So far no optimum amino acid ratio in potato has been established.
Research	Control of Asn levels in tubers Farming practices, e.g. fertiliser regimes Laboratory and field studies with new potato varieties	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 reducing sugar levels. On its own, the reducing sugar concentration of potatoes is not always directly proportional to the acrylamide concentration observed in a fried potato product. The concentration of free Asn and the ratio of free Asn to other free amino acids (of which glutamine is by far the most abundant) should also be considered and may be better indicators of the relative acrylamide risk of different potato varieties. 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 / amino acids levels. In potatoes, the effect of sulphur is uncertain, and any advice on S-fertilization to farmers would be premature based on the studies conducted so far. An increased ratio of other amino acids to Asn results in competition for reactants during the Maillard reaction, potentially affecting the proportion of AA formed in the overall Maillard process. New potato varieties with silenced Asn synthase genes in the tuber have up to 20-fold reduced amounts of free Asn. Heat processed products derived from such tubers show comparable sensorial properties to their conventional counterparts, and expectedly much lower levels of AA. This work is based on lab trials. In field trials in the U.S., new potato varieties with silenced Asn synthase genes in the tuber have up to 5-fold reduced amounts of free Asn. Heat-processed products derived from such tubers show comparable sensorial properties to their conventional counterparts, and expectedly much lower levels of AA (50-75% reduction). As of writing such potatoes are not yet approved for commercial production in the EU or US, therefore the regulatory status should be checked.

Other Considerations

- 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 (colour, flavour, etc.).
- Elimination of defective materials as part of standard good manufacturing practice has proven to be an effective measure to reduce AA in the final product. For example, this can be done through working with suppliers to ensure that in-coming potatoes are checked whilst in storage, and again at plant, for defects (e.g. bruising and damage, viruses such as Spraing/ TRV, fungal diseases, storage rots).

3. Recipe: Other Minor Ingredients (amino acids, calcium salts and co-ingredients)

Development	No success in lowering AA by addition of amino acids and promising results for calcium salts in the laboratory were not confirmed at industrial scale	**Amino acids** In laboratory trials glycine was not successful in lowering AA. **Calcium salts** Despite promising results on laboratory scale, calcium lactate did not give satisfying results when tested on an industrial scale.

4. Recipe: pH

Development	Promising results in the laboratory were not confirmed at industrial scale	Addition of low levels of acids to raw materials has shown synergistic benefits with calcium salts in small-scale pilot trials for crisps. However the same tests for French fries at laboratory scale showed serious sour off-taste. The use of acidulants (acetic, citric acids) and ascorbic acid in the French fry industry proved to be promising at laboratory scale as a mitigation tool. However, their application on the industrial production of French fries did not result in additional AA reductions compared to the standard product. Moreover, great care must be taken in order to avoid sour off-taste.

5. Recipe: Dilution and Piece Size

Commercial Application	Thicker strips reduce AA through the surface area / volume effect	AA is formed on the surface and the surface to volume ratio affects the quantity of AA formed. Decreasing the surface area to volume ratio by creating thicker strips/sticks of potato could be one way of reducing AA. However the strip cut dimension is specified by customers.

6. Processing: Asparaginase

Development	Asparaginase may reduce AA in an optimised lab environment which, however, differs significantly from an industrial setting	Despite the fact that asparaginase reduced the AA content of the final product in preliminary laboratory experiments, the application in the industrial production of frozen parfried French fries could give various results. Some AA reduction as well as no reduction was found during pilot-line or industrial tests, depending on the conditions of application of the enzyme. In a production line of blanched (non-parfried) chilled potato strips longer enzyme-substrate contact time is allowed, which leads to total Asn depletion for the enzyme treated fries after four days of cold storage.

7. Processing: Thermal Input & Moisture

General Considerations

- 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 at a lower product temperature to optimise colour development and minimize AA formation.
- For French fries the finished frying / (oven) cooking of the prefried potato product is done by the professional end-user or by the consumer at home (see guidelines under 'final preparation').

Commercial Application	For French fries, final preparation conditions are key as AA is formed during final cooking	Par-frying does not produce significant levels of AA in the semi-finished product, nor does it determine the level in the final product.

Other Considerations

Effect of reducing the frying temperature on the fat content of the finished product (for example 9 mm fries: lowering the final frying temperature can lead to an increase in fat, when frying to the same colour endpoint). Effect of incomplete cooking on moisture level in products may have a subsequent impact on product quality, shelf life, and/or microbiological damage.

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

Commercial Application	Blanching of potato strips/ pieces prior to processing	The blanching process is the most important tool to control reducing sugars (by equally removing glucose and fructose) from the outer surface layer of the potato strips (UGhent study).
Commercial Application	Strategic dextrose addition	**Dextrose addition** Strategic addition of dextrose (=glucose) after blanching (=leaching out both accumulated natural reducing sugars (glucose and fructose) on the outer surface of potato strips results in lower AA levels in the final cooked product at the same (Agtron) colour. The underlying mechanism is that in reacting with Asn, fructose generates higher AA levels at the same finished frying colour compared to glucose (see Higley et al, 2012; Parker et al, 2012). Rationale for dextrose addition: • Achieving final colour as specified by customer to match local consumer preferences • Giving fries a uniform colour (after final cooking by end user) • Compensating for variability in raw material. Dextrose addition has little relevance on the AA content of the final product (Vinci et al, 2012).
Commercial Application	Disodium diphosphate	**Disodium diphosphate** Addition of disodium diphosphate directly after blanching of French fries is used to avoid discolouration of uncooked strips and has a secondary effect of reducing AA by lowering pH, which inhibits the Maillard reaction.

Development	The addition of di- and trivalent cations has been proposed to reduce the formation of AA	**Calcium salts** The addition of di- and tri-valent cations has been proposed to reduce the formation of AA in several potato products. • The use of calcium salts (calcium lactate, calcium chloride) in the French fry industry proved to be promising at laboratory 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 disodium diphosphates which is generally used to prevent grey discoloration. • The use of magnesium chloride gave rise to serious off-tastes.

9. Processing: Finished Product Colour

Commercial Application	Measure & manage finished (fried) product colour after final cooking to colour specification	A study conducted by Ghent University on the main parameters (reducing sugar content and colour evaluation) linked to AA in the final product, revealed that the best correlation was achieved by colour determination (Agtron process analyser). The cooking instructions on the packaging have been revised to achieve a golden yellow colour for the finished product. Following these revised optimised cooking instructions results in lower AA levels. It has been identified that in some European countries consumer preference for French fries is to have them cooked to a golden brown colour rather than golden yellow. Use of food colourings as an ingredient in industrially produced products could be an effective tool to produce golden brown French fries in some countries. However, there are legal constraints on the use of food colourings in plain potato products in certain regions (e.g. EU). Lighter coloured French fries can be an acceptable product for consumers if other organoleptic properties such as taste and texture are managed.

Other Considerations

As part of standard quality procedures, manufacturers should implement controls which may have an indirect impact upon AA levels in the final product e.g. management of oil quality to ensure cooking temperatures are optimally maintained.
Modification of texture and flavour are not directly suitable as a tool to reduce AA, but are impacted by other interventions.

10. Final Preparation: Consumer and Restaurant Guidance

Commercial Application	Advice to Chefs and consumers	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) colour • when cooking small amounts, reduce the cooking time. Oven products: • do not overcook • cook to a golden (yellow) colour. When cooking small amounts, reduce the cooking time. European Potato Processors Association (EUPPA) developed a specific tool presenting "Good frying Practices" to help consumers and restaurants : **www.goodfries.eu** The US FDA has a webpage listing the ways to reduce AA "Acrylamide: Information on Diet, Food Storage, and Food Preparation", which can be found: : here
Development	"Fresh" prefabricated French Fries 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. 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 10-45 min).

Cereal/Grain Based Products

Products include bread, crisp bread, pretzels, breakfast cereals, and biscuits / bakery wares [classification as defined in the CAOBISCO study 2008, including crackers, semisweet products, pastries, short sweet biscuits, wafers, cakes and gingerbread].

1. Agronomy: Reducing Sugars

General Considerations: Breakfast Cereals

The sugars composition of cereal grains is not a key determinant of AA formation and therefore has not previously been considered relevant to breakfast cereal manufacture.

Commercial Application	Sugars composition of cereal grains is of less impact that the aspragine level in the raw materials	Research confirms that Asn rather than sugars is the key determinant of AA formation in cereal products. Free Asn levels have been found to have a much greater impact than the levels of reducing sugars in wheat. 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 AA formation) and may also be beneficial in reducing AA in products that do not contain added reducing sugars in their recipe. No correlation was found for bread and wheat flours.

2. Agronomy: Asparagine

General Considerations

Asn is the critical component which leads to the formation of AA in cereal products. Research shows that free Asn varies widely within one single variety, growing conditions on individual fields (e.g. impact of sulphur) and between cereal varieties. This is confirmed by University research on heated wheat flour, where it has been has shown that free Asn concentration is the key determinant. Because of the sources of variation (grain type, variety, growing conditions, climate), it is impossible to source wheat or any other grain with controlled low levels of Asn. Moreover, depending on the type of grain used a product has specific characteristics defining the product identity. Consequently, it is not possible to simply replace the grain by another grain without changing the product identity.

For these reasons, more fundamental knowledge is needed concerning the impact of agronomical practices and cereal varieties on Asn level. Therefore, Industry Partners support research proposals that aim to investigate breeding science to control Asn in wheat varieties.

Commerical Application	Farmers should be aware of the importance of maintaining balanced sulphur levels for cereal cultivation[1]	Sulphur-deprived soils have been shown to impact the free Asn concentrations in certain cereal crops considerably. Less sulphur in the soil results in higher Asn levels in the crop and therefore higher risk of AA formation. Cooked wheat prepared from sulphur-deficient flour also impacts the spectrum of aroma compounds, and consequently the organoleptic properties. 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 practical application levels in farming. Even a 25% reduction in Asn would achieve more than most interventions tested to date.

[1] For example, in the United Kingdom, the recommended level of sulphur for wheat cultivation in the UK is 20 kg per hectare – Source: Journal of Experimental Botany, Vol. 63, No. 8, pp. 2841–2851, 2012.

Development	Choice of wheat varieties Lowering the portion of wholegrain and/or bran in products will significantly impact nutritional quality and organoleptic properties of the product	**Breakfast cereals** Choice of wheat with lower free Asn has led to products with lower AA levels However, current experience suggests that specifying low free Asn wholegrain is not yet possible, but that using less whole meal (lower bran and germ) and more endosperm will be effective (as free Asn is more concentrated in the germ/bran) but will significantly compromise the product's organoleptic and nutritional properties. Most breakfast cereals are made from soft wheat. Soft wheat typically has higher protein/amino acid content which provide competing amino acids to the Asn 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. **Cereal based products** Current experience suggests that specifying low free Asn wholegrain is not yet possible, but that using less whole meal and/or bran and more endosperm will be effective (as free Asn is more concentrated in the germ/bran) but will significantly compromise the product's organoleptic and nutritional properties. Reducing the nutritional quality (i.e. less whole meal, fibre and other beneficial nutrients) would negatively impact its intake (in the worst case exacerbating deficiencies) as cereals are a major contributor to the consumer intake. **Crisp bread** 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.

3. Recipe: Raising Agents

Commercial Application	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. Despite changes to flavour, colour 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 colour, 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. 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.

4. Recipe: Other Minor Ingredients

Commercial Application	Replacing fructose with glucose is very effective in reducing AA formation, particularly in recipes containing ammonium bicarbonate	**Short sweet biscuits** Reducing sugars are responsible for many of the characteristic flavours and colour in sweet biscuits. Fructose replacement by glucose retained original quality and texture in several commercial applications; paler colours were, however, acceptable. When glucose-fructose syrups are used, the fructose content should be as low as possible.
	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 AA 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.
Development	Glycine addition impacts sensorial properties Calcium gives variable results and most have adverse flavour effects	**Gingerbread** 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]. Trials at bench scale with added calcium salts gives variable results, most affecting product quality. None have been commercialised.
	Glycine addition provides only a marginal reduction in AA	**Bread** 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 (ca. 16%).
	Glycine addition changes product colour/quality	**Short sweet biscuits** Glycine addition changes product colour and furnishes products of unacceptable quality.

Development	Sugars are usually added after the toast. The impact of malt on AA formation requires further study	**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 AA levels. Added fructose tends to cause severe browning. Honey, glucose, fructose and other reducing sugars are generally used in the sugar coat applied after toasting so they do not influence AA formation. One manufacturer reports that malt, specifically malted barley, has been shown to reduce AA in breakfast cereals. However, the impact of malt requires further study and today the effect cannot be considered as a general rule.
	Addition of calcium chloride requires further study	The addition of calcium and glycine is under investigation at lab and pilot scale. Many breakfast cereals are fortified with Ca^{2+}, and manufacturers could explore the benefit for those not so fortified. A manufacturer reports that calcium chloride has been shown to reduce AA in breakfast cereals at pilot scale. The flavour appears to be acceptable for calcium chloride up to 0.4 to 0.5% of solids. Flavour validation is in progress.
	Addition of glycine is under investigation at pilot scale	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 colour and a bitter taste. Due to the high reaction rate of glycine, even at smallest addition levels, trials to date were not successful to control the effect of glycine on colour, flavour and taste with requirements for product moisture, texture and shelf life: Manufacturers added glycine in the pilot plant with other amino acids (proline & lysine) and found that glycine and proline (but not lysine) did reduce AA but all three amino acids imparted an unacceptable bitter flavour to the product.
	Positive effect of antioxidants not evident	Manufacturers also report about trials with various antioxidants (vitamin C & E) in pilot plant trials, albeit without a meaningful reduction in AA.
	Addition of glycine and calcium impact negatively key quality attributes	**Crisp bread** The level of AA can be affected by glycine at 3% (w/w) resulting in a reduction by approx. 78%. However, the colour may well be affected and an undesirable sweet flavour introduced. Ca^{2+}, whilst only slightly reducing AA, has an adverse effect on the flavour and texture at pilot plant level.

5. Recipe: pH

Development	Addition of organic acids has only been effective when combined with a change to leavening agents, and then with only marginal impact	**Biscuits, crisp bread, gingerbread, breakfast cereals** 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. 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. 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 **crisp bread** 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). Addition of citric acid or salt compounds to **breakfast cereals** has been tested in lab and pilot scales but the palatability of the final product has not been found acceptable. Increasing the pH (by adding NaOH) did reduce AA but also adversely affected the colour and taste. Decreasing the pH (citric acid) during the heat treatment step showed either no or only very limited effect but in any case resulted in unacceptable taste.

6. Recipe: Dilution & Piece Size

Commercial Application	Reducing wholemeal in the recipe affords lower AA in crisp bread. However, impact on nutritional quality and organoleptic properties needs to be assessed	**Crisp bread** If crisp breads 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.
	Piece size	In crisp breads, the thicker the bread, the lower the AA levels. This, however, significantly changes the product characteristics.
	Grain replacement may be an option in some recipes	**Short sweet biscuits** The part replacement of wheat flour by rice flour is an effective measure.

Commercial Application

Using less whole meal and/or bran and more endosperm will significantly comprise organoleptic properties and the nutritional quality of products with a negative impact on consumer intake of whole meal, fibre and other beneficial nutrients

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. Different kind of grains have shown different distribution of asparagine, the pre-cursor for the formation of AA (see table below showing examples of variations: *Source: MAP Milling Project - Measure and control of mycotoxins, pesticides and acrylamide in grain milling sector. Project funded by the European Commission under the research and technological development programme 'Integrating and strengthening the ERA' (2002-2006)*http://toiduliit.ee/Upload/User/File/ettekanded/IGV%20training%20material.pdf)

Cereal grain	AA (ppb)
Rice	< 50
Maize	50-110
Durum	150-460
Wheat	350-500
Oats	~500
Rye	450-880

However, the choice of grain defines the food and therefore it is not possible to simply replace the grain by another grain without changing the whole product and losing the product identity the consumers like.

Other ingredients used in cereal products may contribute to AA. Almonds roasted at relatively lower temperature contain about 10 fold less AA than almonds roasted at higher temperatures. Peanuts and hazelnuts contain less than a fifth of Asn as compared to almonds, consequently the latter yield much less AA. Where baked pieces are used in muesli their recipe should be reviewed alongside the advice for biscuits.

Roasted nuts and dried fruits may contribute to the AA burden

Some dried fruits (e.g. prunes, pears) were reported to contain AA. Tests were therefore made on some ingredients commonly added to muesli 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 certain bakery products)

AA is formed in the hot drying crust of bread. 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.

7. Recipe: Rework

Development	There is evidence at the laboratory 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. In pilot studies with sweet biscuit dough, it has been shown that more AA is formed in biscuits baked from older dough (an increase of approximately 35% over 3 h). The extra AA could be accounted for by the measured increase in free Asn over time. 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. Other work conducted on non-fermented crisp bread has shown no significant effect on the formation of AA in the product.
	No clear indication on the impact of rework	**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.

8. Recipe: Fermentation

Commercial Application	Lower levels of AA in fermented products. Extension of fermentation time in bread may be an option to lower AA levels	**Crisp bread** Some baked products, such as crisp breads 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. Yeast rapidly assimilates Asn and aspartic acid, as well as sugars. Crisp bread, which is mainly produced with yeast, also shows significantly lower AA content for fermented variants versus cold bread (non-fermented variants). In crisp bread manufacture, other factors such as biscuit thickness and baking conditions must be seen in perspective.

	In sweet biscuits, control of dough storage time may be a good practice Use of lower gassing yeast to decompose Asn faster	**Sweet biscuits and crackers** More AA is formed in dough that has been allowed to age (35% increase over 3h), i.e. increase in free Asn in dough over time. Hence, avoid adding "aged" dough. However, no studies on reducing dough hold time in hard sweet biscuits were reported in the latest CAOBISCO survey. Biscuit and cracker dough: 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. However, no studies on increasing fermentation time in crackers were reported in the latest CAOBISCO survey. 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. **Bread** Longer rising time of the dough and the use of special yeast contribute to a lower Asn content.

9. Processing: Asparaginase

Commercial Application	Use of asparaginase is effective in biscuits, cereals, crisp bread, and today is applied to commercial products (e.g. gingerbread, crisp bread, short sweet biscuits, Ready-To-Eat cereals,) with potential also in other biscuit and cereal product types	**Biscuits, gingerbread, and crisp bread** Certain products are today produced with the use of asparaginase without any quality issues, e.g. gingerbread, crisp bread 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. In Fine Bakery Wares, AA reduction due to the use of asparaginase will greatly vary depending on recipe, ingredients used and the moisture content of the products.
	Asparaginase is effective in certain cereal-based snacks (corn and wheat based)	**Cereal/Grain based snacks and brezels** Significant reductions (~70 - 90%) have been achieved through the use of asparaginase in certain cereal dough-based snacks, and are now in use at industrial scale. A minimum residence time dependent on Asn levels is required to achieve maximal reduction.
Research	No significant Asn reduction in a cooked and toasted coarse grain cereal	**Breakfast Cereals** No significant Asn reduction in a cooked toasted coarse grain cereal. Trials at laboratory and pilot scale in collaboration with an enzyme supplier confirmed that asparaginase was not effective for mitigation of AA with breakfast cereal processes used. The breakfast cereal processes use a low moisture content which makes enzyme penetration into the grain or food matrix difficult. Many breakfasts cereal processes use coarse flours or chopped grains which are not readily penetrated by the enzyme.

10. Processing: Thermal Input & Moisture

Commercial Application	Optimisation of thermal input has resulted in a reduction of AA in crisp bread	**Crisp bread** In non-fermented crisp bread, 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 colour, flavour and texture.
	Optimisation of thermal input has resulted in a reduction of AA in breakfast cereals	**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). One Manufacturer reports that for breakfast cereals, this concept was evaluated and results showed that AA correlates with moisture but does not correlate well with colour. Therefore, at constant moisture (for toasted products), AA is expected to be constant under conditions of breakfast cereal processing. However, other Manufacturers report that they evaluated to lower heating temperatures and (separately) shorter heating times and, although both do reduce AA, they adversely affect product colour and taste. Different combinations of heating temperature and time were also examined and it was found that all the combinations that produce an acceptable colour & flavour also have a similar AA level. This suggests that the scope to reduce AA by optimising thermal input may only be restricted to certain breakfast cereal recipe/process combinations. AA content tends to be correlated with moisture content of toasted product (as lower moisture content is usually generated by a higher thermal input), is well correlated with moisture content but manufacturers generally apply both maximum and minimum ranges for moisture as a part of routine quality management, and raising the moisture content tends to compromise shelf-life.
Development	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 colour generation, baking with lidded pans, and using falling oven temperature profiles. 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. 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.

11. Processing: Finished Product Colour

Commercial Application	Colour 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	**Hard sweet biscuits, crisp bread, breakfast cereals, bread** The Maillard reaction, which leads to the production of AA, also produces the colours and flavours which give baked cereal products their essential characteristics. If, though, one was able to produce lighter coloured and less baked products, without increasing the moisture content, the AA level could theoretically be reduced. Colour endpoint is an approach applied for (i) hard sweet biscuits with a reported ca. 10% reduction in AA and lighter colour, and (ii) crisp bread through reduced "final roast" with acceptable reduction in browning. A correlation between AA and the colour in breakfast cereals is a case-by- case situation and could not be established generally. In bread, the endpoint colour does in most cases reflect AA content.

12. Processing: Texture/flavour

Development	Close correlation to moisture, an important organoleptic / quality attribute	**Biscuits** It is unfortunate that the reaction leading to the formation of AA, the Maillard reaction is also that which develops flavour and colour. In some products (e.g. gingerbread) reducing sugars, such as glucose or fructose, are deliberately added so as to achieve particular flavours (and colour). 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 are warranted to assess feasibility and tolerance in terms of product acceptance.

13. Final Preparation: Consumer, Customer and Restaurant Guidance

Commercial Application	Pack instruction / customer information	**Bread, including partial baked products and toasted bread** Bread is only baked to a light colour and acrylamide levels measured are usually low (5-10 ug/kg) in white bread. However, bread may be subjected to further thermal treatment during toasting. It is therefore important to toast bread to a light golden colour. A growing part of industrial bread production is delivered to « bake off ». Those products are only baked partially and then deep frozen. There is no risk of acrylamide formation in the first baking step. The responsible final bake off process in the bakery retail shop is assured through clear user information (time and temperature).

Coffee, roasted grain & substitute

1. Agronomy: Reducing Sugars

Development	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. **Coffee Substitutes mainly based on cereals** Sugar levels in the cereals (e.g. barley) show no correlation to the amount of AA formed during roasting. **Other coffee substitutes (e.g. mainly based on 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.

2. Agronomy: Asparagine

Development	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. As identified during the FoodDrinkEurope/EC Workshop in 2010, 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. **Coffee substitutes mainly based on cereals** The amount of Asn in cereal (e.g. barley) is relatively low (about 30 mg/100g) and will determine the final amount of AA in the roasted cereal product. Roasted cereal products tend to have relatively lower amounts of AA because at the higher temperatures of roasting and longer roasting time AA is degraded rapidly. **Other coffee substitutes (e.g. mainly based on chicory)** The range of free Asn in chicory roots is relatively narrows (40 - 230 mg/100g). Studies at pilot scale show that Asn content of dried chicory is correlated to the formation of AA.

Other Considerations

Other "marginal" pathways not related to free Asn may become important for the formation of AA.

3. Recipe: Other Minor Ingredients

Research	Calcium and magnesium salts are not effective in reducing AA in coffee. Adding calcium or magnesium has been evaluated not to be a tool to reduce AA levels in chicory	**Other coffee substitutes (e.g. mainly based on chicory)** Results from laboratory studies showed a significant reduction in the amount of AA in roasted chicory after 2h soaking in a bath of calcium and/or magnesium salts . A reduction of AA in the range of 40-95% was achieved compared to untreated chicory which was roasted under comparable conditions (i.e. temperature and final colour) Although these preliminary results seem promising, several issues were observed, i.e.: • The treatment showed a significant impact on the products sensorial properties. • Scaled up trials revealed significant microbiological and environmental concerns.

4. Recipe: Dilution & Piece Size

Commercial Application	Recipe modification to accommodate lower percentage of high AA-forming constituents	**Other coffee substitutes (e.g. mainly based on chicory)** Lowering the chicory content (for example by 3%) in the coffee substitutes recipes and partial substitution (e.g. by roasted barley, chicory fibres) achieves a reduction of AA but may have an impact on organoleptic properties of the product.

5. Processing: Asparaginase

<table>
<tr>
<td>Research</td>
<td>Applying asparaginase enzymes in coffee may have application opportunities in particular for steam treated Robusta.</td>
<td>
Coffee
Results from coffee industry laboratory/pilot plant trials indicate a reduction potential for AA in the range of 5 – 45 % depending on (i) the green coffee type, (ii) conditions of the green coffee enzyme treatment process when compared to an untreated coffee which has been roasted under the same conditions. Recent trials with Robusta coffee indicate that an enzyme process alone is more likely to result in a reduction at the lower end of the range whereas a combination with a green coffee steaming process may result in reductions at the higher end of the range.
In any further assessment it is recommended to include the following findings and considerations:

Trials as referenced above were conducted under laboratory /pilot plant conditions only. Scaling up will require an assessment of the suitability of treatment conditions as applied in laboratory/pilot plants for application at commercial plant scale. In this assessment, the food safety aspects (in particular the microbiological safety) and impact on sustainability (in particular energy use) of the finished product should be taken into account as well.
A potential green coffee enzyme process is a new and an additional process for roast coffee and soluble coffee manufacturing. This process cannot be integrated into the existing roast coffee manufacturing processes and will accordingly require new plants and facilities, except for potential capacities in existing decaffeination and green coffee steam treatment facilities after required adaptation to the enzyme process.
The acceptability of the impact on flavor has to be assessed on an individual case-by-case basis depending on green coffee type, the percentage of the enzyme treated coffee in the total blend and the specific quality targets of a product. Results from earlier trials have shown that the taste of coffee may get significantly and negatively influenced by the treatment process. In particular the Arabica coffee beans, which are known for their appreciated contribution to taste and aroma in the final coffee brew, have been seen to be severely affected (treatment leading to off-taste profiles), as a result of and depending on the process conditions used. Applying the asparaginase process to Robusta green coffees, which for flavour modification purposes are steam treated in parallel, are more likely to be acceptable from a flavour impact perspective due to the intentional stronger flavour change from the steaming process and the typically limited percentage of this component in the total coffee blend of a product.

Other coffee substitutes (e.g. mainly based on chicory)
Laboratory scale trials reveal up to 70% reduction in AA in raw chicory when treated for several hours in an asparaginase solution at appropriate temperature. However, this treatment has a significant impact on final product quality and microbiological safety.
</td>
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6. Processing: Thermal Input & Moisture

Development	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. Different to most other food categories, the AA concentration in coffee decreases with increasing thermal input/darker roasting. 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. Trials on new/alternative roasting technologies have been conducted. A steam/pressure roasting pilot plant unit resulted in a reduction potential of up to 10% in comparison with a conventionally roasted sample of similar quality - not indicative of a significant mitigation opportunity. **Coffee substitutes mainly based on cereals** In cereals (e.g. barley) AA is formed at temperatures above 120°C, with a maximum at 150°C. Above 150°C, the AA level decreases with continued roasting. Final finished product levels are at only 30-40% of the maximum level, final concentration being dependent on the target degree of roast. Darker roasting in general tends to reduce the AA level but this parameter needs to be fixed in narrow ranges to achieve the target flavour profile. **Other coffee substitutes (e.g. mainly based on chicory)** In chicory, AA is formed at temperatures >130°C, with a maximum at ca. 145°C. Above 150°C, rapid decrease in AA due to process-related loss. Colour development >150°C due to caramelisation (degradation of sucrose). Decreasing the roasting temperature and concomitantly increasing the roasting time, favours the loss of AA. Possible mitigation therefore involves over-roasting (by increasing temperature above 150°C and/or roasting time). This strong heat treatment has a significant impact on the final product's quality (colour development, change of taste) and on its acceptance by the consumer (especially regarding to traditional products such as roasted chicory or mixes of chicory and coffee).

Other Considerations

Results on coffee have led to the conclusion that only very limited process options are available to reduce the AA level without affecting the quality /consumer acceptance of the product. Moreover, when mitigation measures for AA are considered, checks should be made to ensure that they will not result in an increase in other process contaminants such as furan.

7. Processing: Pre-treatment

Development	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 typical green coffee moisture of 10-12% to approximately 7%) did not show an impact on the AA level in the roasted product. Decaffeination: trials showed that roasting of decaffeinated green coffees (covering the commercially important decaffeination processes) resulted in AA levels of same magnitude as roasting of corresponding untreated coffees when roasted under comparable roasting conditions. **Coffee substitutes mainly based on cereals** Soaking is a common process applied to cereals prior to roasting. However, soaking has no significant impact on AA generation.

8. Processing: Finished Product Colour

Commercial Application	Colour is an important process control point and linked to the sensory properties of the product	**Coffee** Colour 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). **Coffee substitutes mainly based on cereals** Colour is an important indicator of roasting degree and directly related to the organoleptic properties of the product. **Other coffee substitutes (e.g. mainly based on chicory)** Colour development results mainly from caramelisation of sugars, and colour is an important end-point of roasting degree and attribute for consumer acceptance.

Other Considerations

In the case of coffee, roasting to darker colour 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) and formation of other undesirable products under extreme roasting conditions need to be considered.

9. Processing: Texture/flavour

General Considerations

In the case of coffee, organoleptic properties are finely tuned by careful selection of green coffee blends, roasting conditions, and processing technologies. 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 and subsequently the consumer acceptance.

10. Final Preparation: Consumer and Restaurant Guidance

General Considerations

Typical brewing equipment transfers AA almost completely into the beverage. The cup/beverage concentrations for roast coffee and soluble coffee are similar. Espresso brewing may however show lower transfer rates due to specific extraction conditions. 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).

Baby biscuits, infant cereals and baby foods other than cereal based foods

1. Recipe: Other Minor Ingredients

Commercial Application	In certain technologies, avoid adding ingredients that may contribute to increasing reducing sugars in the recipe	**Infant cereals** In roller dried infant cereals, whole grain, reducing sugars addition to the mix (e.g. fruits, honey, fructose), leads to a higher amount of AA in the final product.

Commercial Application	Recipe may impact AA in jarred baby foods	**Baby foods other than cereal based foods** Products containing sweet potatoes and prunes are of greater risk, due to relatively higher amounts of AA precursors. In the case of prunes, optimization of heat treatment in the drying process (reduction of time/temperature conditions) can be effective.
	Recipe has a major impact on final AA level	**Baby biscuits** Generally applicable to biscuits, whole grain, reducing sugars in the recipe may lead to higher amounts of AA in the final product.

Important considerations

Any changes (lowering) of thermal input to reduce AA in baby foods must be carefully considered due to (more severe) microbiological risks.

2. Processing: Asparaginase

Commercial Application	Asparaginase is very effective in certain infant cereal processes	**Infant cereals** Different technologies are employed to manufacture infant cereals, e.g. extrusion, roller drying. Most ingredients contain large proportion of cereal flours, and recipes are usually characterized by high water content in the wet mix hydrolysis step, enabling the use of asparaginase. Provided the incubation / residence time, temperature, and mixing conditions are controlled, asparaginase addition can result in a significant decrease (up to 80%) of Asn.

Commercial Application	Asparaginase can be effective in baby biscuits	**Baby biscuits** Asparaginase can be added at the wet-mixing step under controlled time/temperature conditions. Depending on the technology, reductions in the range of 30-60% have been observed in baby biscuits/snacks.

3. Processing: Thermal Input and Moisture

Commercial Application	Thermal input is an effective measure but within certain limits	**Baby foods other than cereal based foods** Mitigation techniques identified to decrease AA is optimization of heat treatment: i.e. reduction of time/temperature conditions. However, a minimum heat treatment is required, i.e. to ensure commercial sterility.

Important considerations

Any changes (lowering) of thermal input to reduce AA in baby foods must be carefully considered due to (more severe) microbiological risks.

Further Reading

General

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Potato based products

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