The CIAA Acrylamide "Toolbox"

Confédération des industries agro-alimentaires de l'UE
Confederation of the food and drink industries of the EU

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Summary

The CIAA "Toolbox" reflects the results of several years of industry cooperation to understand acrylamide formation and potential intervention steps. Its aim is to provide brief descriptions of the intervention steps evaluated and, in many cases, already implemented by food manufacturers and other partners in the food chain. This approach is intended to assist individual manufacturers, including small and medium size enterprises with limited R&D resources, to assess and evaluate which of the intervention steps identified so far may be helpful to reduce acrylamide formation in their specific manufacturing processes and products. It is important that they assess the suitability of proposed mitigation steps in the light of the actual composition of their products, their manufacturing equipment, and their need to continue to provide consumers with quality products consistent with their brand image and consumer expectations. It is anticipated that some of the tools and parameters will also be helpful within the context of domestic food preparation and in food service establishments, where stringent control of cooking conditions may be more difficult.

A total of 13 parameters, grouped within the four major Toolbox compartments, have been identified. These parameters can be applied selectively by each food producer in line with their particular needs and product/process criteria. In addition, the stage at which the different studies have been conducted, i.e. laboratory, pilot, or in a factory setting (industrial), are aligned to the potential mitigation measures. This approach ensures that all pertinent tests and studies are captured independent of their immediate applicability to commercial production.

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

Background

In April 2002, authorities, food industry, caterers and consumers were surprised by the unexpected finding that many heated foods contained significant levels of acrylamide, a substance known until then only as a highly reactive industrial chemical, present also at low levels for example in tobacco smoke. The toxicological data suggested that this substance might be – directly or indirectly – carcinogenic also for humans. Recent assessments by JECFA, WHO and SCF confirmed that such a risk cannot be excluded for dietary intake of acrylamide, but did not confirm that this would be relevant at the low dietary exposure level compared to other sources of exposure, e.g. occupational. At the EU level, progress in the research on acrylamide has been shared openly and regularly through forums such as the "Acrylamide Stakeholder meetings", held in October 2003 and January 2005. A formal presentation was given to the Commission Working Group on 16 September 2005 when the Toolbox concept was supported by the Commission and national authority representatives: CIAA was urged to publish the first official version of the Toolbox without delay. The present text is the second edition incorporating latest developments and knowledge, including the key points that were presented at the joint CIAA/EC Workshop on Acrylamide held in Brussels, March 2006 [1]. The Toolbox has also been assessed by Heatox and is consistent with its recent findings.

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

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

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

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. Several intermediates in the "dry" Maillard reaction cascade have been proposed, such as 3-aminopropionamide. The role of this compound as a key transient intermediate has recently been confirmed through isotope labelled experiments in a cheese model (Schieberle, Koehler and Granvogl, CIAA/EC Workshop on Acrylamide, Brussels, March 2006).

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

In many cooking processes, the Maillard cascade is the predominant chemical process determining colour, flavour and texture of cooked foods, based on highly complex

[1] The proceedings of the Workshop will be published in a special edition of *Food Additives and Contaminants.*

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 crucial: i.e. the combination of temperature and heating time to which the product is subjected.

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 dependent on natural variations in raw materials.

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

Methods of Analysis and Sampling

Today, many laboratories offer sensitive and reliable methods to analyse acrylamide in a wide range of foods. Issues with the extractability of acrylamide in certain food matrices were raised by Eriksson & Karlsson, showing that a high extraction pH may significantly enhance the yield of acrylamide versus extraction under neutral pH conditions [*LWT* (2006), 39, 392-398]. This observation suggests that the methods developed to date may underestimate the concentration of acrylamide in certain foods. Work done by independent research groups [Goldmann *et al*. (*Food Additives and Contaminants*, 23(5), 437-445, 2006) and Hajslova *et al*. (Presentation, Cost 927 Workshop, Napoli, 2006)] confirmed, however, that the “additional” acrylamide released at high pH is not due to the improved extractability of the analyte from the food matrix, but rather an extraction artefact formed due to the decomposition - under extreme pH conditions - of certain hitherto unidentified precursors. Consequently, the choice of reliable of analytical methods is of crucial importance.

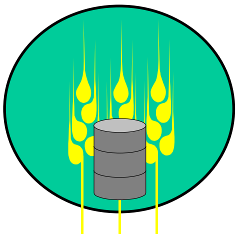
The main challenge for the analyst is the high variability of the products. This starts from the natural variability of a given raw material – any potato can be considered as “individual” with noticeable differences in composition and thus potential for acrylamide formation. Slight differences in product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

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

Definition and Use of the Toolbox Parameters

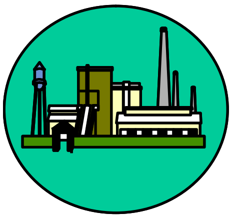
The summaries describing the acrylamide reduction various tools developed by industry are intended to be generic. This is necessary to take account of the differences between product recipes, designs of processes and equipment, and brand-related product characteristics even within a single product category.

The following 13 parameters, grouped within the original Toolbox compartments, have been identified:

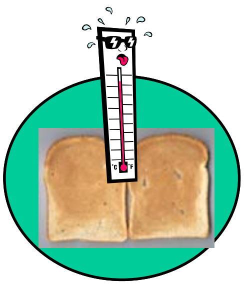


- Agronomical
- Sugars
- Asparagine

- Recipe
- Ammonium bicarbonate
- Other minor ingredients (e.g. calcium salts, glycine)
- pH
- Dilution
- Rework



- Processing
- Fermentation
- Thermal input and moisture control
- Pre-treatment (e.g. washing, blanching, decaffeination, asparaginase)



- Final Preparation
- Colour endpoint
- Texture/flavour
- Product storage/shelf-life/consumer preparation

An example of how the parameters may fit into the Toolbox compartments is illustrated in Figure 1.

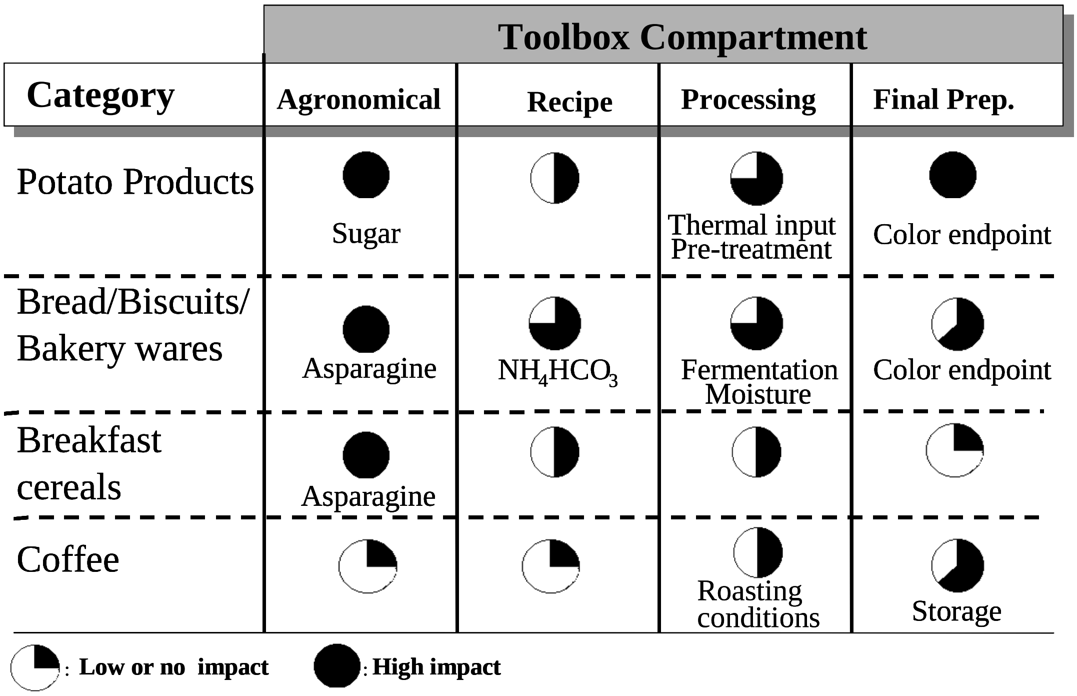


Figure 1. Examples of "generic" parameters that may impact acrylamide formation in the different food categories (non-exhaustive list). Note, the degree of shading of the circles is an indicator of the impact this tool may have and is not to be interpreted as a quantitative measure.

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

The summaries in this document also specify the level of experience available for a proposed intervention:

> *** Lab Scale:** This indicates that - for the categories mentioned - only experimental work has been done to assess the impact of the proposed intervention. Large scale industrial application has either not yet been done or has failed in the specific context. This does not necessarily mean that the concept would not function for other applications.

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

*** Industrial:** 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.

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

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

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

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 will be applied (see Figure 1). These individual interventions may lead to an overall reduction of the desired mitigation effect. Particularly in products with highly complex recipes like biscuits, it is very difficult to predict the "real life" impact of a given measure.

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

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

*** 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.

*** 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, such as the enzyme asparaginase, 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.

*** Risk/Risk and Risk/Benefit Positioning**: Changes in product composition and/or process conditions may have an impact on other product parameters with potential health or nutritional implications:

- 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).
- 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 significantly.

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

*** Variability:** As indicated in the context of the analytical considerations, 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 is needed to permit comparisons: single analyses are nearly always insufficient to evaluate the effect of an intervention for a product.

Abbreviations used

AA: acrylamide; **Asn**: asparagine; **Caobisco**: Association of the Chocolate, Biscuits and Confectionery Industries of the EU; **EC**: European Commission; **GAP**: Good Agricultural Practice.

Agronomical: Sugars

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

Industrial Minimising sugars has been part of standard manufacturing practice Storage temperature N-fertilisation may impact reducing sugar content	Reducing sugars are one of the key reactants for the formation of AA [1]. The sugar content of the tuber correlates well with the AA concentration in the product especially if the fructose/Asn ratio is < 2 [2]. Controlling sugar is currently the primary measure employed by the industry to reduce AA levels in crisps and French fries [3] by: ▪ Selection of potato varieties with low reducing sugars (target less than 0.3 % sugar on a fresh wt basis, subject to seasonal variability) ▪ Lot selection based on reducing sugars content or colour assessment of a fried sample. ▪ Controlling storage conditions from farm to factory (e.g. temp. > 6°C identified as good practice for long storage [4], use of sprout suppressants following GAP, reconditioning at higher temp. (e.g. ambient) over a period of a few weeks). These measures are implemented throughout the industry. Future opportunities include: ▪ Breeding new potato varieties with lower reducing sugar content and/or less cold sweetening effect. ▪ Further optimise the agricultural practices to reduce sugars and Asn. The nitrogen fertiliser regime appears to influence the reducing sugar concentration of the potato tuber, i.e. increased reducing sugars (60 – 100%) upon lowering the field N-fertilisation [5].

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

Pilot scale, Industrial Sugars composition of cereal grains is not a key determinant of AA formation	The sugars composition of cereal grain has not previously been considered relevant to breakfast cereal manufacture. Measurements for four soft wheat varieties in 2004 found 1 to 1.3% dry wt of total reducing sugars as glucose (0.41 - 0.58%); fructose (0.17 - 0.2%) and maltose (0.36 - 0.55%). Sucrose was at 0.5 - 0.65%. AA formation showed no relation to total reducing sugars or to individual sugars concentration. Subsequent research confirms that Asn rather than sugars is the key determinant for cereal products.

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

Pilot scale
No correlation to AA formation

Coffee
Sugar levels in the green beans (robusta, arabica) show no correlation to the amount of AA formed during roasting.

Chicory
Inulin and sucrose at approx. 67g/100g dried chicory, and reducing sugars approx. 1.9g/100g (dry wt). These amounts increase substantially during roasting. No relationship between sugar levels and AA formation during roasting [6].

Other considerations

Potatoes:

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

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[5] De Wilde et al., (2006) *J. Agric Fd Chem*. 54, 404-408.
[6] CIAA Technical Report "Acrylamide Status Report December 2004" A summary of the efforts and progress achieved to date by the European Food and Drink Industry (CIAA) in lowering levels of acrylamide in food. Brussels, Jan 2005. (http://www.ciaa.be).

Agronomical: Asparagine (Asn)

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

Lab scale Currently no practical means available to control Asn level in potatoes	Asn, an important amino acid for plant growth, is the other key reactant for AA formation. In potatoes, Asn is the most abundant free amino acid, typically 0.2 - 4% dry wt, 20 - 60% of total free amino acids. Asn levels do not correlate to sugar levels. So far no control of Asn levels in potatoes has been established. Potential leads being explored include: ▪ Breeding of lower Asn varieties ▪ Impact of farming practices (e.g. fertiliser regimes) on Asn levels ▪ Impact of storage on free Asn levels.

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

Pilot scale Selection of crop varieties with lower Asn may provide a viable option to lower AA levels in finished products	Asn is the critical component which leads to the formation of AA in cereal products [1]. Free Asn within and between cereal crops varies widely. Year to year variations from one harvest to another are considerable and more fundamental knowledge is needed concerning the impact of agronomical practices and cereal varieties on Asn level. Work on these issues is in progress and publication is likely within the year. Notably, sulphur-deprived soils have been shown to impact the free Asn concentrations in certain cereal crops considerably. Choice of wheat with lower free Asn has led to products with lower AA levels [2]. The major soft wheat varieties in use in the UK are, perhaps by chance, those with lowest Asn concentration. In France, more variation is seen and selection of soft wheat varieties has been applied. Current experience suggests that specifying low Asn wholegrain is not yet possible, but that using less whole meal and more endosperm will be effective but will change the product's organoleptic and nutritional properties. [3]

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

Pilot scale No mitigation options through crop selection due to narrow window of free Asn. Contribution of marginal pathways not yet clarified.	**Coffee** Free Asn concentrations in green coffee beans lie within a very narrow range, typically from 30–90 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 [4, 5]. As identified during the CIAA/EC Workshop [6], 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

potential contribution of minor pathways (e.g. thermolytic protein cleavage) in this product category.

Agronomic aspects not adequately studied and are considered long-term

Chicory

The range of free Asn in chicory roots is relatively narrow (40 - 230 mg/100g). Studies at pilot scale show that Asn content of dried chicory is correlated to the formation of AA [2].

Other considerations

Impact of reducing free Asn on plant health and consumer nutrition.

In coffee, other "marginal" pathways not related to free Asn may become important for the formation of AA [4].

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[4] Stadler, R.H. & Scholz, G. (2004) *Nutrition Reviews*, 62:449-467.

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[6] CIAA/EC Acrylamide Workshop, Brussels, March 2006. Conclusions of the Coffee WG.

Recipe: Ammonium bicarbonate

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

Not applicable

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

Pilot scale and Industrial Feasibility of reducing NH_4HCO_3 in recipes for *finished products* has yet to be established, and could lead to significant increase in sodium levels	**Biscuits** Replacing NH_4HCO_3 with alternative raising agents is a demonstrated way to lower AA in certain products and on a case-by-case basis [1]. 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. Experiments have shown that NH_4HCO_3 can promote the production of AA in gingerbread [2]. The NH_4HCO_3 contributes to the AA formation by indirectly catalysing the degradation of sugars to produce reactive carbonyls. Although a recent study has shown that complete substitution of NH_4HCO_3 with $NaHCO_3$ can reduce AA by approx. 70% in a sweet intermediate biscuit [3, 4], the complete substitution in a typical semi-sweet finished product increased the sodium content from 144mg/100g to >500mg/100g, and also produced a much more dense product. Work on gingerbread and shortcrust pastry has shown that complete replacement of NH_4HCO_3 with $NaHCO_3$ leads to significant textural and sensorial changes [5].

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

Not applicable

Other considerations

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

References

[1] Review of Acrylamide Mitigation in Biscuits, Crackers and Crispbread. CAOBISCO, May 2006, available upon request at caobisco@caobisco.be

[2] Amrein, TM., Schönbächler, B., Escher, F., Amado, R. (2004) *J. Agric Food Chem*. 52: 4282-4288.

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[4] Graf, M., Amrein, T., Graf, S., Szalay, R., Escher, F., Amado, R. (2005) *Food Sci & Technol*.,(2005), 39, 724-728.

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Recipe: Other minor ingredients

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

Lab & Pilot scale

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

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.

- Some AA reduction has been has been found in laboratory scale trials where potato slices have been soaked in solutions of glycine or glutamine [1]

Deactivation of Asn by:

- Addition of di- and tri-valent cations has been proposed in the patent literature to reduce the formation of AA in fabricated potato chips [US2004126469, US2004058045, US20050507538]. In French fry and potato nugget models, addition of $CaCl_2$ (100 – 20,000 mg/kg) and soaking led to a 24 – 96% decrease in AA content [2]. The mechanism by which Ca^{2+} effects a reduction in certain applications has not yet been clarified and further studies are warranted.

In a potato cake model, the combined treatment of citric acid and glycine (each 0.39% in the recipe) had an additive effect on 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. [3]

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

Industrial

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

Biscuits

Reducing sugars are responsible for many of the characteristic flavours and colour in sweet biscuits, and their replacement with sucrose causes severe loss of colour. However, very promising results have been achieved by replacing fructose with other reducing sugars such as glucose. When glucose-fructose syrups are used, the fructose content should be as low as possible [4].

Gingerbread

In pilot trials, glycine addition (1 % in the recipe) decreased AA content ~2.5 fold and enhanced browning, but with a clear impact on the sensorial properties of the product [5].

Bread

Adding Ca^{2+} lowers AA in certain products by up to 30%, and adding Ca^{2+} to the tin releasing agent may be an option as most AA is formed in the crust [6].

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

Lab scale
Addition of Ca^{2+} or Mg^{2+} to certain bakery products/ breakfast cereals and of glycine to crispbread/ breakfast cereals may be options

[1, 7].

Breakfast cereals
In breakfast cereal production, manufacturers generally use sucrose and small amounts of malt in the cereal itself because reducing sugars darken the cereal too much. Honey, glucose, fructose and other reducing sugars are used in the sugar coat applied after toasting so they do not influence AA formation [3, 4]. In the light of today's knowledge, one would consider carefully any proposal to add reducing sugars at the cooking stage. Several trials show no effect of malt on AA formation, it seems likely that Asn content dominates at the levels of malt used.
Many breakfast cereals are fortified with Ca^{2+}, and manufacturers could explore the benefit for those not so fortified. No significant effect of calcium addition has been seen by those manufacturers who report trials. 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.
Crispbread
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 [4]. Ca^{2+} may have an effect but this needs to be studied.

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

Not applicable

Other considerations

Crispbread: addition of glycine may impart a bitter note.
Potatoes:
- o Environmental impact
- o Glycine: nutritional impact to be studied
- o Calcium chloride: corrosion of the equipment, nutritional benefits versus risks
- o Frying oil quality/ citric acid

- Bakery products for diabetics: greater potential of AA formation if sucrose is replaced by fructose [5]
- Calcium dosing and nutritional aspects to be considered
- Potential formaldehyde formation from glycine to be considered

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Recipe: pH

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

See section "Processing: pre-treatment".

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

Lab, Pilot & Industrial

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

Biscuits, Crispbread, Gingerbread

Laboratory experiments with an intermediate product (semi-sweet biscuit) have shown that reducing pH by addition of citric acid can lead to 20-30% reduction of AA in the intermediate product [1].

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) [2]. In crispbread and biscuits, the pH has an impact on the organoleptic properties of the final product.

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

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

Not applicable

Other considerations

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

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Recipe: Dilution

Potato (Potato products for frying, roasting, baking, but mainly French fries and potato crisps)	
Industrial Partial replacement with ingredients lower in key reactants	For some preformed/reconstituted products, partial replacement of potato components by ingredients lower in key reactants reduces AA formation potential, and has been implemented, e.g. use of cereals with lower Asn amounts than potato (e.g. wheat, rice, maize) in the recipe [1].

Cereal (e.g. bread, crispbread, biscuits/bakery wares, breakfast cereals)	
Pilot scale Individual studies required, depending on recipes; impact on nutritional quality and organoleptic properties needs to be assessed	**Crispbread** If crispbreads are produced with cereal grains that are low in Asn, then consequently products low in AA are expected. It is possible to dilute the Asn-containing material in certain cases. Depending on the choice of the diluting material, this may change the product composition and characteristics considerably [2]. **Breakfast cereals** All of the major grains may be used in breakfast cereals and some grains yield more AA than others within a common process. Wheat yields markedly more AA than maize, oats or rice. However, the choice of grain defines the food.

Coffee and Coffee Mixtures (e.g. roast and ground, soluble coffees, coffee mixtures)	
Industrial Recipe modification to accommodate lower % of high AA-forming constituents	**Chicory** Lowering the chicory content by 3% in the recipes for coffee surrogates and partial substitution with, for example roasted barley, achieves marginal reduction but has an impact on organoleptic properties [1].

Other considerations

Cereals: Asn is more concentrated in the bran so use of refined grain may reduce AA. However, the fibre and other beneficial nutrients of bran would be lost, and the product characteristics would also change [3].
Potatoes: implication for consumer acceptance and products characteristics.
Denomination of product may change based on the dilutions with other cereal grains.

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Recipe: Rework

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

Not applicable

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

Industrial

Knowledge inadequate to assess impact of rework on AA formation

Biscuits

Based on studies conducted in Germany, rework in certain bakery wares may have an impact on the amount of AA present in the final product [1]. For example, a 16% reduction of AA in gingerbread has been shown without rework. Other work conducted on non-fermented crispbread has shown no significant effect on the formation of AA in the product [2].

Breakfast cereals

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

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

Not applicable

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Processing: Fermentation

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

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

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

Lab, Pilot & Industrial Lower levels of AA in fermented products. Extension of fermentation time in bread may be an option to lower AA levels	**Biscuits** Some baked products, such as crispbreads and crackers, can be made from fermented doughs so as to develop specific textures and flavours. Compared to similar non-fermented products, the level of AA in the fermented variants is generally lower [1]. Yeast rapidly assimilates Asn and aspartic acid, as well as sugars. Crispbread, which is mainly produced with yeast, also shows significantly lower AA content for fermented variants versus coldbread (non-fermented variants). In crispbread manufacture, other factors such as biscuit thickness and baking conditions must be seen in perspective [2]. **Bread** Lab scale trials have shown that extended yeast fermentation may be one way to reduce AA content in bread [3]. Yeast preferentially removes Asn and a study in the UK showed a 50% reduction of AA after 1h fermentation time [4]. This observation warrants further study.

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

Not applicable [5]

Other considerations

Fermentation leads to increased glycerol and in combination with lower pH (addition of acids) may favour the formation of 3-MCPD [6].
Long reaction times and compatibility with industrial processing and effect on finished product quality need to be considered.

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Processing: Thermal input & moisture control

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

Industrial Thermal input controls AA formation in the finished product	**Crisps** Thermal input rather than temperature alone is critical to controlling product characteristics. This needs to take account of temperature and frying times and processing equipment. ▪ Different solutions to optimise thermal input to manage AA have been implemented in line with existing processing equipment. ▪ Vacuum frying might offer the opportunity for producing reduced AA crisps if only potatoes with high sugar levels are available. For manufacturers that use high temperature flash frying, rapid cooling helps to reduce AA formation.
For French fries, final preparation conditions are key	**French fries** Par-frying does not produce significant levels of AA in the semi-finished product, nor does it determine the level in the final product. It is the final preparation that has the controlling influence. See section "Final Preparation" for positive action taken.
Lab scale Par-frying and high temp drying using dry steam	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 [1]. Industry is already frying to a maximum end moisture content to make an acceptable product.

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

Industrial Optimisation of thermal input has resulted in a reduction of AA in crispbread	The formation of AA during the baking of cereal products is closely related to the combination of moisture content and baking temperature/time (thermal input). If products could be baked to the same moisture, but with less colour development, less AA would be formed even though longer baking times would be required [2, 3]. **Crispbread** In non-fermented crispbread, reduction in process temperature and oven speed reduced AA by approx. 75 %. The most important impact is coming from securing that the end humidity is as high as tolerable from a quality point of view. However, other products may suffer significant changes to colour, flavour and texture [3, 4, 5]. **Breakfast cereals** There is one report of a reduction in AA for a baked breakfast cereal when a two-zone oven with a cooler section replaced a

single zone oven. Multi-zone ovens and toasters are the norm for large manufacturers and they have not found benefit without significant changes to organoleptic properties. Two manufacturers have recently experimented with baking conditions and found that the conditions associated with minimum energy use are also associated with least AA formation.

Lab & Pilot scale
Alternative baking technologies

Bread
Alternative technologies for baking such as infrared has afforded up to 50% reduction of AA in the crust [6].

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

Lab & Pilot scale
Roasting technologies beyond existing ones are still in the knowledge-building / exploratory phase: preliminary data does 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 [4, 5, 7].

Different to most other food categories, the AA concentration in coffee decreases with increasing thermal input/darker roasting. The hypothesis is that at higher temperatures, as applied during coffee roasting, reactions leading to the depletion of AA dominate towards the end of the roasting cycle. These reactions are as yet not understood, but the hypothesis is supported by studies in model systems that show an increase and subsequent decrease of AA over temperature, explained by potential polymerisation or reaction of AA with food components [8].

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

Chicory
AA is formed at temperatures > 130°C, with a maximum at 145°C. Above 150°C, rapid decrease in AA due to process-related loss. 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.

Other considerations

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

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

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Processing: Pre-treatment

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

Industrial
For French fries, blanching is the most important tool to control AA.
Addition of sodium acid pyrophosphate reduces pH and thus lowers potential of AA formation

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

During the last stage of blanching sodium acid pyrophosphate is added. In this way the pH is lowered as an additional measure to reduce AA formation.

Lab & Pilot scale
Crisps: Variable success with washing or pH control

Crisps
Different solutions to control key reactants, have been implemented, with various success, dependant on existing processing equipment:

- Washing
- pH control

Asparaginase may reduce AA in reconstituted dough-based products

Reconstituted Potato Products
Laboratory and pilot plant trials have shown that asparaginase significantly reduces the levels of AA in potato dough based products [1]. However, the same effect is not found in sliced or chipped potato products. If the potato cells remain intact, asparaginase will have difficulty in reaching the substrate (Asn).
Currently the enzyme is not commercially available and not approved for use in food applications.

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

Lab & Pilot scale
Use of asparaginase shows great promise in certain products

Biscuits
Asparaginase has been shown to be effective in hard doughs, but much increased enzyme addition was needed to achieve a reduction in AA for short doughs (only 5 - 20% reduction in AA compared to 90% in some hard semi sweet doughs). Nevertheless this has a high potential for AA reduction especially in high moisture, neutral pH systems at elevated temperatures [2].

Trials with a rice-based cereal product showed that low amounts of asparaginase (10 - 50 mg/kg) in the cereal soup can lower the free Asn amount in the product by > 90% over a relatively short period of time (5 – 15 min). This intervention will be further pursued once the enzyme has attained approval

	status for use in food and sensory tests can be conducted.
Coffee and Coffee Mixtures (e.g. roast and ground, soluble coffees, coffee mixtures)	
Lab & Pilot scale Use of asparaginase to be assessed at industrial scale, taking into consideration applicability and impact on quality/sensorial attributes	**Coffee** Pre-drying of green beans: green coffee dried to lower moisture content prior to roasting (from a typical green coffee moisture of 10-12% to approximately 7%) did not show an impact of the initial green coffee moisture on the AA level in the roasted product [3]. Decaffeination: trials showed that roasting of decaffeinated green coffees (covering the commercially important decaffeination processes) results in AA levels of same magnitude as roasting of corresponding untreated coffees when roasted to comparable roasting conditions. Asn reduction: enzymatic processes claiming an AA reduction of 10-90% has been filed as a patent [4]. An example provides data achieving a reduction of approx. 27% on roast coffee basis. Potential impact on quality due to the additional processing steps and due to significant removal of Asn not addressed. **Chicory** Asn reduction: as above.

Other considerations

Current hurdles with regard to availability of the enzyme on a commercial scale and permitted use of asparaginase for food trials, i.e. need urgent clarity on the safety, regulatory & patent situation.

Potato products:

- the applicability of asparaginase needs to be evaluated in terms of process feasibility
- effect of acids on corrosion of processing equipment, and on product characteristics
- environmental impact.

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Final Preparation: Colour endpoint

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

Industrial Cook to a golden yellow colour. In-line elimination of dark crisps	**French fries** The cooking instructions on the packaging have been revised to achieve a golden yellow colour for the finished product. **Crisps** Elimination of dark coloured crisps by in-line optical sorting has proven to be an effective measure to reduce AA.

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

Industrial 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	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, but without increasing the moisture content, the AA level could theoretically be reduced. [1, 2, 3] In some cases a darker colour may be associated with less AA e.g. some breakfast cereals [1, 4]. In bread, the endpoint colour does in most cases reflect AA content [5].

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

Pilot scale & Industrial 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) [1, 2, 6]. **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 and formation of other undesirable products under extreme roasting conditions.

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Final Preparation: Texture/flavour

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

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

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

Pilot scale Close correlation to moisture, an important organoleptic attribute	**Biscuits** It is unfortunate that the reaction leading to the formation of AA, the Maillard reaction, is also that which develops flavour and 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 warranted to assess feasibility and acceptance tolerance [1]. In crispbread, the thicker the bread, the lower the AA levels. This, however, significantly changes the product characteristics.

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

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

Other considerations

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

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Final Preparation: Product storage/shelf-life/consumer preparation

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

User advice French fries: cook until a golden yellow colour	**French fries** Follow exactly the product specific cooking instructions on the packaging. Frying products: ▪ Cook at maximum 175°C for prescribed time ▪ Do not overcook ▪ Cook to a golden yellow 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
Lab Scale “Fresh” prefabricates may have higher sugar contents toward end of product shelf life	A study performed in Switzerland has shown that “fresh” prefabricates of 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. Residual enzyme activity (α-amylase) may slowly release reducing sugars during cold temperature storage [1]. This warrants further investigation. **Crisps** Storage does not influence the level of AA in the finished products.

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

Not applicable	**Biscuits and breakfast cereals** Storage does not influence the level of AA in finished products.

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

Lab scale AA is not stable over longer storage times. Studies into mechanisms of reduction are planned and the knowledge may open avenues of mitigation, provided	**Coffee** Brewing: typical household and non-household brewing equipment transfers the AA of the roast coffee almost completely into the beverage. Espresso brewing may however shows lower transfer rates due to specific extraction conditions [2]. Soluble Coffee vs. Roast Coffee: similarly, in soluble coffee AA is efficiently extracted and concentrated into the final soluble coffee. After preparation/brewing the cup/beverage levels for roast coffee and soluble coffee are similar due to different typical recipes (with ca. 5-7g for roast coffee resp. ca. 2 g of soluble coffee per cup). Shelf-life: AA amounts in packed roast coffees are

sensory qualities are maintained

significantly decreasing over storage time [2, 3]. The storage temperature also has an impact. Studies are in progress to determine the fate of AA in roasted coffee as this may help identify compounds that selectively interact with AA. However, an immediate mitigation opportunity is not expected as freshness is one of the key quality parameters for coffee [4].

Chicory

Similar behaviour in coffee mixtures as compared to pure soluble coffees [3].

Other considerations

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

Finish frying (catering)/ Optimization of the fryer temperature program is important to ensure that temperature does not drop below a given limit after loading the fryer [5].

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