Review

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Current issues in dietary acrylamide: formation, mitigation and risk assessment

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Abstract

Acrylamide (AA) is known as a neurotoxin in humans and it is classified as a probable human carcinogen by the International Agency of Research on Cancer. AA is produced as by-product of the Maillard reaction in starchy foods processed at high temperatures (>120 °C). This review includes the investigation of AA precursors, mechanisms of AA formation and AA mitigation technologies in potato, cereal and coffee products. Additionally, most relevant issues of AA risk assessment are discussed. New technologies tested from laboratory to industrial scale face, as a major challenge, the reduction of AA content of browned food, while still maintaining its attractive organoleptic properties. Reducing sugars such as glucose and fructose are the major contributors to AA in potato-based products. On the other hand, the limiting substrate of AA formation in cereals and coffee is the free amino acid asparagine. For some products the addition of glycine or asparaginase reduces AA formation during baking. Since, for potatoes, the limiting substrate is reducing sugars, increases in sugar content in potatoes during storage then introduce some difficulties and potentially quite large variations in the AA content of the final product. Sugars in potatoes may be reduced by blanching. Levels of AA in different foods show large variations and no general upper limit is easily applicable, since some formation will always occur. Current policy is that practical measures should be taken voluntarily to reduce AA formation in vulnerable foods since AA is considered a health risk at the concentrations found in foods.

Keywords: acrylamide; formation; risk assessment; mitigation

INTRODUCTION

Acrylamide (AA) (2-propenamide, CAS No. 79-06-01) is a colorless and odorless crystalline solid. Owing to its capability to polymerize it is commonly used as flocculant for water purification, a constituent in cosmetics and as a sealing adjuvant in tunnel construction. In relation to the latter use the adverse health effects of AA were pinpointed in Sweden in 2002. AA was propelled into the spotlight in 2002 when the Swedish National Food Administration and the University of Stockholm reported considerably high levels of this probably carcinogenic compound[1] in commonly consumed foods such as bread, coffee, potato crisps, French fries and many others.[2]

AA induces tumors in several organs in mice[3] and rats[4,5] and exerts reproductive[6,7] and neurotoxic damage.[7–9] After dietary consumption AA is rapidly absorbed from the gastrointestinal tract and widely distributed to the tissues.[10] In the liver AA is metabolized to glycidamide (GA), which is more reactive towards DNA and proteins.

AA is formed mainly from free asparagine and reducing sugars during high-temperature cooking and processing of common foods, principally through Maillard reactions.[11]

It is evident that the main sources of human dietary exposure to AA are those of fried potato (∼272–570 μg kg^{-1}), bakery products (∼75–1044 μg kg^{-1}), breakfast cereals (∼149 μg kg^{-1}) and coffee (∼229–890 μg kg^{-1}).[12]

Considering that AA is a genotoxic compound, the margin of exposure (MOE) criterion is used for its risk assessment. The MOE is defined as the benchmark dose divided by the dose from exposure. In all cases the MOE to dietary AA of different aged populations in several worldwide zones is below 10 000 and in some cases reduced to <200; hence dietary AA exposure may be considered as of health concern.[13–19] Based on this relatively low MOE, several studies have established an association between the exposure to AA and human cancer; however, most studies show no clear association between the AA dietary intake and an increase in human cancer.

This could be explained by the fact that, since huge variations in AA occur in some food products, dietary exposure estimates based on food frequency questionnaires may be an uncertain parameter for actual AA exposure. In this sense, AA biomarkers (AA haemoglobin adducts and glycidamide haemoglobin adducts) directly measured in the blood of humans may provide a more accurate estimate of AA exposure.

Despite the lack of association between estimated dietary AA exposure and cancer, current available information has convinced numerous scientific committees and regulatory agencies worldwide that exposure to AA by humans should be limited to the lowest possible level. Most recently, in March 2010,

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the European Chemical Agency added AA to its list of substances of 'very high concern'.[20]

Even though the food safety world reacted rapidly to the discovery of AA, the application of AA reduction technologies in the food industry remains a pending issue.[21]

To reduce dietary AA, the food industry faces the challenge of changing processes and/or product parameters without compromising the taste, texture and appearance of their products.[22,23] In this sense, AA reduction would be greatly facilitated by the development of potato and wheat varieties with lower concentrations of free asparagine and/or reducing sugars, as well as of best agronomic practice to ensure that concentrations are kept as low as possible. The breeding of these crops would help to avert raw material substitution and enable the food industry to comply with the regulatory system as it evolves without the need for additives or potential costly changes in processes.[24]

The present review includes critical issues of dietary AA. Its toxicity is presented to show the relevant problems that AA intake may provoke in human beings. Additionally, AA formation is discussed since its better understanding is crucial to reduce the AA occurrence in foods. Analytical techniques for its quantification and current AA monitoring are presented. AA risk assessment based on exposure estimation and multiple-generation animal toxicity studies are presented. Finally, different mitigation technologies are discussed which may be applied in the food industry.

TOXICOLOGY

AA toxicity

AA has neurotoxic effects and is also carcinogenic in experimental animals.[25] Human studies have revealed that occupational exposure to AA produces symptoms of peripheral neuropathy.[26] Studies using hemoglobin adducts as biomarkers of occupational exposure of Swedish tunnel workers to a grouting agent have shown that AA resulted in mild and reversible peripheral nervous system symptoms.[27] Likewise, a previous study in China involving 71 workers in a small AA factory showed that longer exposure to AA resulted in cerebellar dysfunction, followed by neuropathy.[28]

The Joint Expert Committee on Food Additives (JECFA) of the World Health Organization (WHO)[29] evaluated the most sensitive adverse non-carcinogenic effect to be the morphological changes in nerves of rats with a no-observed-adverse-effect level (NOAEL) of 200 $\mu g\ kg_{bw}^{-1}\ d^{-1}$.

AA is classified by the International Agency for Research on Cancer[1] as probably carcinogenic to humans. Previously, the dose–response relationship of AA in relation to cancer risk assessment has been based on tumor incidences from the 2-year carcinogenicity studies of Friedman *et al.*[4] and Johnson *et al.*[5] The same 2-year study was repeated by the National Center for Toxicological Research (NCTR)/National Toxicology Program (NTP)[30,31] and used by JECFA for AA risk assessment.[29] The study in which mice and rats were treated with AA in drinking water again found cancer in the thyroid and mammary gland of male and female F344 rats at a dose concordant with those found in the previous 2-year studies in rats.[4] Additional tumor sites observed in the new study were heart schwannomas and pancreatic islet tumors in males. The new study also reported cancer in lung, Harderian gland, forestomach, mammary and ovary of $B6C3F_1$ mice. JECFA considered that the available epidemiological data were not suitable for a dose–response analysis. Therefore, the assessment was based on the available studies in laboratory animals, i.e. the NCTR/NTP 2-year bioassay of AA in rats and mice. Here the lowest range of $BMDL_{10}$ values was observed for the Harderian gland in $B6C3F_1$ mice treated with AA (BMDL – bench mark dose level – is the dose estimated from a dose–response curve of observable effects corresponding to a particular level of response, e.g. 10% ($BMDL_{10}$), and which include a 95% confidence limit). As humans have no equivalent organ, the significance of these benign mouse tumors in the Harderian gland is difficult to interpret with respect to humans. However, in view of AA being a multisite carcinogen in rodents, the evaluation committee was unable to discount the effect. JECFA considered it appropriate to use 180 $\mu g\ kg_{bw}^{-1}\ d^{-1}$ (the lowest value in the range of $BMDL_{10}$ values) for tumors in the Harderian gland of male mice and 310 $\mu g\ kg_{bw}^{-1}\ d^{-1}$ for mammary tumors in female rats as the points of departure (PODs).

AA toxicokinetic and dynamics

After dietary consumption, AA is rapidly absorbed from the gastrointestinal tract and widely distributed to the tissue.[10] In the liver it is metabolized to an epoxide glycidamide by the liver metabolizing system CYP2E1.[32] The metabolite glycidamide is far more reactive toward DNA and proteins than AA.[33] Studies on rats, mice and humans suggest efficient human metabolism of AA to glycidamide.[34] However, in humans there is considerable variability in the extent of AA conversion to glycidamide, which appears to be related to inter-individual variability in the amount of liver CYP2E1. Regarding the haemoglobin adduct levels of AA and glycidamide in humans compared to animal species Vikstrom *et al.*[35] found that the AA haemoglobin adducts is about five times higher and glycidamide haemoglobin adducts nearly two times higher after dietary AA exposure compared to the levels in F344 rats exposed as in cancer studies;[35] for glycidamide haemoglobin adducts the difference in biotransformation between humans and rats is regarded as modest. Fennell *et al.*[10] concluded that internal exposure to glycidamide in humans was two to four times less than in the mouse. When applying water with 0.5, 1 and 3 mg kg^{-1} of AA to five volunteers 34% of the amount was recovered as total metabolites in the urine within 24 h. Fuhr *et al.*[36] gave volunteers a meal containing 0.94 mg AA and recovered 60% of the administered dose as urinary metabolites within 72 h. Glycidamide may be further metabolized by epoxide hydrolase to glyceramide[37] or by conjugation to glutathione, or it may react with proteins, including haemoglobin, or with deoxyribonucleic acid (DNA). AA is extensively conjugated with glutathione to form a mercapturic acid, *N*-acetyl-*S*-(2 carbamoylethyl)-L-cysteine,[32] in all species examined and is oxidized to its corresponding sulfoxide in humans only.[38]

The molecular mechanism of AA toxicity is not fully elucidated. It is demonstrated that the weak electrophilic AA forms covalent adducts with the nucleophilic cysteine thiolate groups located within active sites of presynaptic proteins, causing inactivation of the proteins and thereby impaired neurotransmission (chemically the α,β-unsaturated carbonyl of AA forms an electrophilic site at the β-atom, at which site the molecule can form Michael-type adducts with nucleophiles).[39,40]

LoPachin and Gavin[40] characterizes AA as a type-2 alkene, a chemical class that includes structurally related environmental pollutants (e.g. acrolein) and endogenous mediators of cellular oxidative stress (e.g. 4-hydroxy-2-nonenal). They hypothesize that the environmental type-2 alkenes humans are exposed to may act synergistically with endogenous unsaturated aldehydes, causing increased risk of diseases that may be triggered by oxidative stress (the complex chemical explanation appears in the reference). They propose that cumulative environmental exposure to type-2

alkenes may pose a human health risk. The perspective of this study is their approach, assessing not only the molecular effects of one substance, in this case AA, but the cumulative effect of a class of chemically related substances.

MECHANISM OF FORMATION

The Maillard reaction, in the presence of asparagine, has been shown to be the main pathway for AA formation in a wide range of foods processed at high temperatures.[41–44]

As shown in Fig. 1, AA formation starts with the reaction of a carbonyl compound (a reducing sugar) with the amino acid asparagine, resulting in the corresponding *N*-glycosyl conjugation and the formation of a decarboxylated Schiff base (after dehydration at high temperature).[42] After its decarboxylation, the Schiff base may lead after decomposition directly to AA and an imine or followed by hydrolysis to aminopropionamide and carbonyl compounds. In this respect, it should be noted that aminopropionamide may also yield AA after the elimination of an ammonia group.[45]

AA is mainly formed during heat processing (>120 °C) of foods – primarily those derived from plant origin such as potato and cereal products.[42,44,46] Stable isotope-labeled experiments have shown that the backbone of the AA molecule originates from the amino acid asparagine.[42,44] Asparagine alone could in principle form AA by direct decarboxylation and deamination, but the reaction is inefficient, with extremely low yields.[45] However, asparagine in the presence of reducing sugars (a hydroxycarbonyl moiety) or reactive dicarbonyls furnishes AA in the range up to 1 mol% in model systems.[47]

On the other hand, non-asparagine routes leading to AA have been published over the past years.[48,49] However, these non-asparagine pathways may be considered as of marginal importance because studies in potato- and cereal-based foods have demonstrated the importance of asparagine by effectively reducing AA through the use of the substrate-selective enzyme asparaginase.[20]

Factors affecting AA formation

Most important for AA formation in the Maillard reaction is the presence of its precursors in raw materials, e.g. reducing sugar such as glucose and fructose, and amino acids in the form of free asparagine, and the magnitude of the combined temperature and time load.[50] Although Maillard reaction is the main mechanism of AA formation, depending on the type of food raw material the reaction may present differences in terms of the limiting reactant. In this sense, some genetic factors as well as environmental conditions may also affect the level of AA precursors. Additionally, the processing conditions and water activity of foods may also influence AA formation.

Influence of AA precursors

For potato products the limiting substrates are in general reducing sugars, which vary considerably between cultivars, growing season and storage conditions. But also the content of asparagine has an influence, although less than the reducing sugars.

The levels of reducing sugars and free asparagine in raw potatoes are greatly influenced by the genotype of the potato.[22,51] Additionally, the content of sugars in potatoes is also affected by the storage temperature, being most prominent at the conventional storage temperature (4 °C).

At 4 °C the reducing sugars glucose and fructose markedly increase according to potato variety. Current industrial practices in the potato-processing sector already consider the selection of potato varieties with low reducing sugar content, and a storage temperature of about 8 °C.[22]

The opposite limiting substrate applies to cereal products where the potential for AA formation is mostly determined by the asparagine content of the flour.[49] In general, the content of asparagine is higher in whole-grain flour than in the sifted fractions. Therefore whole-grain products may contain a higher content of AA.[50,52] The AA formation in cereals depends also on the cultivar; however, there is less variation between the varieties and cereal types and no variation induced by storage. In this respect, it was found that rye flour contained 0.41–0.44 g asparagine kg^{-1}, spelt 0.06–0.12 g asparagine kg^{-1} and wheat 0.05–0.25 g asparagine kg^{-1}.[53] Due to elevated asparagine contents, AA in rye products was generally higher than in wheat and spelt products. Fertilizers (calcium ammonium nitrate, ammonium sulfate etc.) applied to cereal products have an impact on amino acids and thus on AA. Furthermore, the time of fertilizer application may play a crucial role.[54] Sulfur and nitrogen availability has been shown to be particularly important. Sulfur deprivation causes a dramatic increase in grain asparagine levels.[50] An opposite situation is produced by nitrogen levels, with increasing nitrogen availability causing grain asparagine levels to rise. Some researchers have shown that nitrogen fertilization resulted in elevated amino acid levels, resulting in increasing AA levels from 11 to 56 µg kg^{-1}.[53]

AA is also formed during roasting of coffee beans, a Maillard reaction process where at the same time the color and the aroma of the coffee beans are produced. The precursors of AA formation are asparagine, which is the amino acid with the second highest concentration in green coffee beans, and carbohydrates, of which sucrose is present in the highest amount.[55] Sucrose is found in concentrations up to 9% dry weight in green coffee beans and during roasting it splits into the reducing sugars glucose and fructose. Asparagine is the limiting precursor for AA formation and is found in higher amounts in Robusta coffee compared to Arabica coffee,[55] implicating higher AA amounts in Robusta compared to Arabica coffee.[56]

Melanoidins are biochemically active polymers that are formed in large amounts during coffee roasting and contribute to the brown color and antioxidant capacity of coffee. Coffee melanoidins have been shown to have a direct influence on the fate of AA under heating, while the effect was not observed at room temperature, which indicates that melanoidins may modulate the reaction pathway of AA formation and elimination during coffee roasting.[57]

Influence of temperature and water activity

In addition to the precursors, AA formation in foods will depend on the heating conditions (heat, time) and water activity (a_w) of the products.[58]

With increasing temperature the reaction rate of AA formation increases in most cases.[59–61] However, the formation of AA in coffee peaks some time before the coffee is ready roasted and the concentration in the roasted coffee is somewhat lower than at the peak level. AA boils at 193 °C and typical roasting temperature is 210–250 °C. Hence some elimination by evaporation or degradation takes place during the roasting and, for that reason, dark-roasted coffee contains less AA than medium-roasted coffee.[62,63]

On the other hand, AA formation from asparagine/glucose model systems was found to increase with decreasing a_w

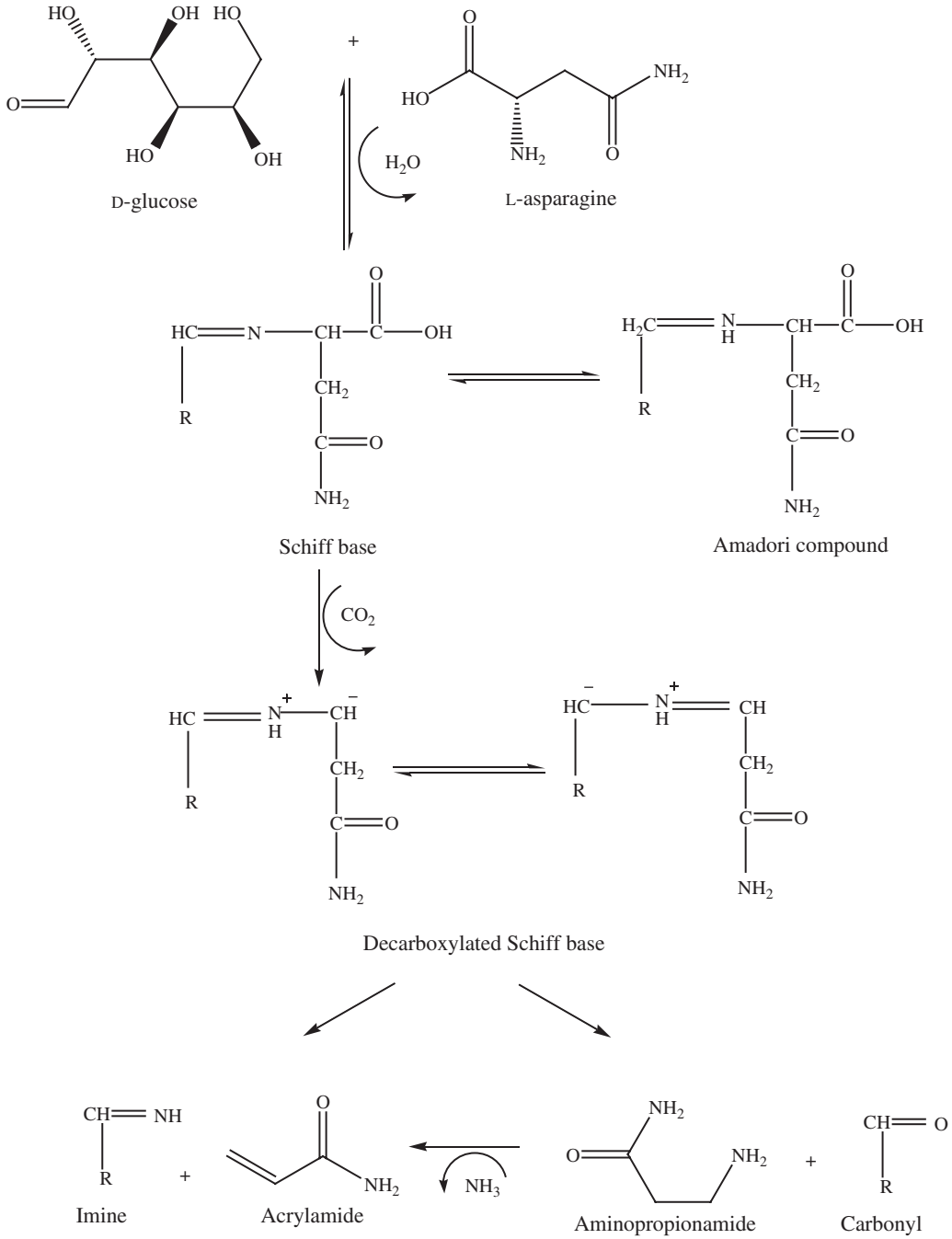

Figure 1. Acrylamide formation by Maillard reaction in heated foods (adapted from Hedegaard *et al.*[141]).

(0.71–0.33). The energy of activation, as estimated from the temperature dependence of the initial rate, increased with decreasing a_w despite a higher rate of formation of AA at low a_w.[60]

Regarding this last finding, contradictory results were found by Robert *et al.*[64] and Mestdagh *et al.*[65] in lower a_w (0.07–0.22) asparagine/glucose and potato powder model systems, in which a_w did not seem to be a critical parameter for AA formation. These differences may be explained since, for the Maillard reaction, intermediate a_w have shown to induce the highest reaction rates because in the intermediate range all reactants are dissolved. A decrease in the free water content will inevitably affect the mobility and viscosity of the system, and consequently changes in AA formation under this lower range of a_w will be less noticeable.[66]

ANALYSIS OF AA

When the occurrence of AA in food was discovered in Sweden in 2002 a liquid chromatographic–tandem mass spectormetric (LC-MS-MS) method for quantification was introduced, the principle of which most later LC-MS/MS methods are based on. The method involves extraction with water and clean-up using solid-phase extraction (SPE).[2,43] A review was published that summarized and presented the state of the art of the analytical chemistry, formation and mitigation recipes of AA in various food matrices.[67] Chromatographic methods enable fast, accurate and reproducible determination of AA. The most widely used methods are based on LC-MS-MS or gas chromatography–mass spectrometry (GC-MS).

Determination of AA using LC-MS-MS may avoid derivatization and has the advantage of rather high sensitivity and stability. Using ultraperformance liquid chromatography (UPLC)-MS/MS or UPLC–time of flight (TOF)-MS may further reduce sample analyses and labor time. GC-MS after bromination is the best GC approach so far, because this method is a relatively mature coupled technique with adequate sensitivity and multiple ion confirmation. Application of GC-MS/MS or coupling to high-resolution MS would lower the detection limit of certain foods even further to 1–2 μg kg^{-1}.[68]

An inter-laboratory study on the LC-MS/MS and the GC-MS methods for AA in bakery ware and potato products in the 20–9000 μg kg^{-1} range found the performance of the HPLC-MS-MS method to be superior to that of GC-MS.[69]

Besides MS detection methods HPLC–diode-array detection (DAD), HPLC-UV detection, GC–electron capture detection (ECD), capillary zone electrophoresis (CZE) immuno-enzymatic tests and, recently, electrochemical biosensors have been introduced.[70,71] Immuno-enzymatic tests are based on selective binding of antigens to be quantified by antibodies. The electrophoretic techniques require short time of analysis and have a high resolution power. As examples, Preston *et al.*[72] developed an immunosorbent assay for AA with 3-mercaptobenzoic acid which conjugated to the carrier protein thyroglobulin. The limit of detection (LOD) in water samples was 66 μg L^{-1}. Quan *et al.*[73] combined enzyme-linked immunosorbent assay with chemiluminescence detection using the marker luminol and obtained good recoveries for realistic spike levels in cereal and potato foods. The LOD was 19 μg L^{-1}. Highly specific biosensors may interact only with one substance contained in a complex matrix. They do not require expensive equipment and may in the future replace conventional methods of AA quantification in food. The most sensitive biosensors for AA analyses of food so far[70] may be a voltammetric sensor based on the formation of adducts between hemoglobin and AA.[74] The formation of AA–hemoglobin adducts changes the structure of the electroactivity in hemoglobin immobilized on the surface of the electrode, which generates a response in the voltamperometric biosensor. The LOD in potato crisps was as low as 1.2 10^{-10} mol L^{-1}. However, the challenge with biosensors is to ensure stability of the biomaterial of the sensor, affecting both the accuracy and precision of the method.

Near-infrared spectrometry (NIR) and computer vision-based image analysis broaden the method selection for the analysis of AA or for controlling AA levels in food production. Since several studies reported a good linear relationship between browning and AA accumulation in chips[75] and in model systems,[46] image analysis of browning may act as an indirect measure of AA concentration as an online process control tool for the frying and baking industry.[76] The possibility of using online NIR monitoring of AA in potato crisp production was also studied. Although the accuracy of the method was modest it may still be of industrial importance to separate crisps with high AA content.[77]

MONITORING AND RISK ANALYSES

Biomarker studies and epidemiological studies

Since the awareness of human AA exposure and the relatively low MOE for a genotoxic compound were established, studies have been made on possible links between exposure to AA and human cancer. The exposure data used include either studies on dietary AA based on food consumption questionnaires/total diet studies or biomarkers such as AA haemoglobin adducts and glycidamide haemoglobin adducts.

Hogervorst *et al.*[78] found an association between dietary AA estimated from food frequency questionnaires and increased risks of postmenopausal endometrial and ovarian cancer in 2589 Dutch women. Also, in the USA, Wilson *et al.*[79] found that endometrial cancer was increased for high-AA consumers assessed among 88 672 women. Furthermore, Bongers *et al.*[80] found indications that AA may increase the risk of multiple myeloma and follicular lymphoma in men. For never-smoking men, the higher risk (HR) for multiple myeloma per 10 μg AA d^{-1} increment was 1.98 (95% confidence intervals: 1.38, 2.85).

Many studies have been conducted on the relationship between estimated dietary exposure and cancer risk indicating that AA exposure was not associated with cancer risk. No significant association was found between AA exposure and, for example, bladder cancer,[79] gastrointestinal cancer,[81] brain cancer,[82] lung cancer,[83] thyroid cancer[84] and prostate cancer.[85] Pelucchi *et al.*[86] made a review and meta-analysis of 25 epidemiological studies on exposure to AA and human cancer. They found a lack of increased risk of most types of cancer from dietary and occupational exposure to AA. The main association that requires further study was kidney cancer, and a prospective study on excess kidney cancer in population groups with high AA exposure is suggested.[86] In 2012 Lipworth *et al.*[87] reviewed conjectured associations between dietary acrylamide exposure and cancer based on more than 30 epidemiological studies. They concluded that a high level of dietary AA was not a risk factor for breast, endometrial or ovarian cancer, and that dietary AA intake failed to demonstrate an increased risk of cancer. The evidence was based, for example, on the absence of a positive association between smoking (causing higher AA exposure compared to dietary exposure) and ovarian or endometrial cancer. Neither did occupational AA exposure result in an association to occurrence of cancer in 696 AA workers followed from 1970 to 2001.[88]

However, the lack of association between estimated dietary AA exposure and cancer may not exclude any association, as most epidemiological studies may not be sensitive enough to detect a small increase in risk, i.e. the studies may not have sufficient statistical power to show any significant association. Furthermore, due to huge variations in the occurrence of AA in some food products, such as French fries, dietary exposure estimates based on food frequency questionnaires may be a relatively uncertain parameter for actual AA exposure. A more accurate estimate of AA exposure is expected to be provided by measuring AA biomarkers directly in the blood of humans in epidemiological studies. The measured adduct level represents a steady-state level from continuous exposure to AA over the previous 120 days, which is the average life span of a red blood cell.[10] In a Danish study of 374 breast cancer cases and 374 controls, after adjustment for smoking, an association was found between AA hemoglobin adduct levels and estrogen receptor-positive breast cancer with an estimated incidence rate of 2.7 for a 10-fold increase in AA hemoglobin concentration.[89] Estrogen-sensitive breast cancer mortality was also evaluated in relation to AA biomarkers.[90] Among 24 697 women of a Danish cohort, 420 developed breast cancer before 2001 and 110 died before 2009. Of the non-smokers, higher concentrations of glycidamide hemoglobin adducts were associated with a higher breast cancer mortality (HR) (95% confidence interval (CI): 1.63 (1.06–2.51)). For AA hemoglobin adduct, the tendency was similar but only significant for those with estrogen receptor-positive tumors (HR (95% CI): 1.31 (1.02–1.69)). Hence AA exposure may be related to breast cancer mortality and this may especially concern the endocrine-related type of breast cancer.

Another biomarker case–control study with 263 incidents of ovarian cancer in Dutch nurses showed no evidence of association with the cancer and AA and glycidamide hemoglobin adducts.[91]

Vesper *et al.*[92] studied AA adducts in a 2003–2004 7166-sample cohort of the US population. AA hemoglobin adducts varied from 3 to 910 pmol g^{-1} hemoglobin and glycidamide hemoglobin adducts from 4 to 756 pmol g^{-1} hemoglobin. They found high variability among individuals but modest variability between subgroups of the population.

AA risk assessment

Dietary exposure to AA has been estimated for several populations and subpopulations. Most of these studies are based on national consumption data and national monitoring data of the AA contents in thermally processed foods. Examples of exposure estimates are compiled in Table 1. The European Food Safety Authority[12] has estimated dietary AA exposures based on mean food contents of 10 366 AA results from 24 European countries in 2007–2009 and national consumption data.

For genotoxic compounds where no tolerable daily intake is recommended, MOE is used for the risk assessment. A MOE above 10 000 for a compound that is both genotoxic and carcinogenic is considered of low health concern.[93] The MOE estimated in Table 1 is based on the carcinogenic effect of AA in mammary glands with a $BMDL_{10}$ of 310 μg kg_{bw}^{-1} d^{-1}.[93] The AA exposures of high consumers are often two to three times that of the average consumers, and that of smaller children often two to three times that of the adults. The MOE are all below 10 000 and in some cases reduced to <200; hence the exposures are of food health concern.

The excess cancer risk from AA exposure has been estimated in several studies. Dybing and Sanner[19] estimated a lifetime excess cancer risk of 6×10^{-4} at an average exposure of 0.46 μg AA $kg_{bw}^{-1}d^{-1}$.[25] This should be seen in the context that the minimum excess cancer risk reported is one in a million (1×10^{-6}) Doerge *et al.*[94] estimated an excess lifetime cancer risk for average AA consumers to be in the range of $1–4 \times 10^{-4}$.[30] Chen *et al.*[95] modeled that if the AA content in French fries is higher than 168 μg kg^{-1} the estimated cancer risk for adolescents aged 13–18 years in Taiwan would be higher than the target excess lifetime cancer risk for high consumers (95th percentile), the excess cancer risk being 3.8×10^{-6} to 1.9×10^{-5} for boys and 3.0×10^{-6} to 1.5×10^{-5} for girls.[95]

AA monitoring and risk management

Since 2007 the European Commission has recommended monitoring and investigating the levels of AA in food.[96–98] As AA is a genotoxic compound, no threshold level exists below which food safety concerns can be disregarded. For that reason the ALARA ('as low as reasonably achievable') principle should be followed with regard to levels of AA in food. In their latest recommendation the Commission recommends investigations into the production and processing methods for products exceeding the indicative values, which are set for most food categories except products for home cooking and other products.[98] The indicative values are intended to indicate the need for an investigation; hence they are not safety thresholds and enforcement should be made based on risk assessments.

European AA monitoring data compiled by EFSA for the monitoring years 2007–2010[12] and the corresponding indicative values – however, only applicable from 2011 – appear in Table 2. The trend analysis based on 13 162 AA data shows only a few changes in AA levels (Table 2). At main food category level, a 'common European trend' was a decrease in AA levels for 'processed cereal based foods for infants and young children' and an increase for 'coffee and coffee substitutes'. Although some optimization has been made by the industry following, for example, the FoodDrinkEurope AA toolbox[99] or Codex guidelines on how to

Table 1. Estimation of dietary exposures and margins of exposures (MOE) based on the carcinogenic effect of acrylamide in mammary glands with $BMDL_{10}$ 310 μg kg_{bw}^{-1} d^{-1} (WHO, 2011)

Population group	Mean exposure (μg kg_{bw}^{-1} d^{-1})	High exposure (95th percentile[a]) (μg $kg_{bw}^{-1}d^{-1}$)	MOE mean exposure	MOE high exposure	Study
WHO estimate	1	4	300-310	75-78	WHO 2011
Belgium, adults	0.36–0.39	0.95–0.99	827	320	EFSA[15]
Brazilian, children11–17 yr	0.04	0.77[b]	7750	403	Arisseto *et al.*[13]
Denmark, adults	0.21	0.46	1442	678	Petersen *et al.*[142]
Denmark, children 4–14 yr	0.39	0.89	803	348	Petersen *et al.*[142]
Finland, adult men 25–44	0.41	0.87[c]	756	356	Hirvonen *et al.*[17]
Finland, children 3 yr	1.01	1.95[c]	307	159	Hirvonen *et al.*[17]
France adults	0.43	1.02	721	304	Sirot *et al.*[16]
France, children 3–17 yr	0.69	1.8	449	172	Sirot *et al.*[16]
Germany, adults	0.31–0.34	0.79–0.83	939	383	EFSA[15]
Ireland, adults	0.59	1.75[c]	525	177	Mills *et al.*[14]
Norway	0.48	1.53	674	203	Dybing and Sanner[19]
Spain, adults 1	0.42–0.45	1.09–1.13	721	279	EFSA[15]
Spain, adults 2	0.55–0.57	1.18–1.23	554	256	EFSA[15]
Spain, boys 11–14 yr	0.534		581		Delgado-Andrade *et al.*[18]
UK, adults	0.61	1.29[c]	508	240	Mills *et al.*[14]
USA, adults	0.4	0.95[b]	775	326	FDA[143]

[a] Unless otherwise stated.
[b] 90th percentile.
[c] 97.5 percentile.

Table 2. AA levels (μg kg^{-1}) monitored from 2007 to 2010 and indicative values (applicable from 2011) for AA occurrence in different foods

Food	Indicative value (μg kg^{-1})	2007			2008			2009			2010		
		No.[a]	Mean[b] (μg kg^{-1})	90 perc. (μg kg^{-1})	No.[a]	Mean[b] (μg kg^{-1})	90 perc. (μg kg^{-1})	No.[a]	Mean[b] (μg kg^{-1})	90 perc. (μg kg^{-1})	No.[a]	Mean[b] (μg kg^{-1})	90 perc. (μg kg^{-1})
Biscuits													
Crackers	500	*27*	237	755	*22*	168	365	*39*	172	504	*64*	178	303
Gingerbread		*458*	387	1074	*395*	355	863	*326*	359	970	*207*	415	1187
Infant biscuit/rusk	250	*79*	174	440	*106*	94	200	*70*	88	203	*46*	86	175
Other biscuits etc.	500	*222*	309	672	*340*	196	476	*353*	180	393	*100*	289	640
Wafers	500	*33*	230	478	*48*	256	645	*85*	206	491	*37*	389	880
Bread													
Crisp bread		*198*	232	480	*93*	228	590	*161*	208	400	*54*	249	665
Bread, soft	150	*176*	75	169	*259*	53	110	*182*	46	69	*150*	30	63
Breakfast cereals	400	*144*	149	333	*166*	155	318	*191*	139	275	*174*	138	293
Cereal-based baby food	100	*65*	69	220	*69*	31	80	*55*	41	38	*82*	31	60
Coffee													
Instant	900	*52*	229	530	*42*	298	660	*51*	551	873	*15*	1123	2629
Not specified		*32*	455	869	*45*	615	898	*9*	679	2929	*9*	441	1800
Roasted	450	*175*	256	519	*280*	197	346	*187*	235	389	*103*	256	462
Substitute coffee		*50*	890	2713	*76*	1033	2392	*32*	1594	3400	*24*	1350	3300
Other products													
Muesli and porridge					*26*	33	81	*72*	58	89	*14*	80	104
Unspecified		*259*	242	608	*274*	120	360	*160*	148	663	*161*	293	707
Potato products													
Potato crisps	1000	*293*	551	1200	*532*	580	1298	*414*	639	1514	*242*	675	1538
French fries	600	*648*	356	742	*536*	277	570	*501*	342	640	*256*	338	725
Home-cooked potato products													
Deep fried		*32*	395	1140	*34*	229	588	*44*	220	549	*64*	198	568
Not specified		*97*	272	623	*99*	213	430	*134*	253	612	*25*	270	707
Oven baked		*8*	365	941	*121*	256	601	*71*	333	782	*28*	690	1888

[a] Number of individual samples analyzed for each food category.
[b] Values based on an upper bond scenario.
Reference: EFSA.[12]

mitigate AA in food,[100] the trends as assessed by EFSA[12] show that during this period it is not significantly affecting the general occurrence of AA in food. The indicative values were exceeded in the case of 3–20% of samples in different food categories based on the 2010 monitoring data. The major food categories contributing to AA exposure for adults were fried potatoes, coffee and soft bread, whereas for adolescents and children they were fried potatoes, soft bread and potato crisps or biscuits.

MITIGATION TECHNIQUES

Efforts by the scientific community have contributed to the identification of potential routes to reduce AA levels in foods and, consequently, AA intake.[58] However, the major challenge is to reduce AA levels in foods as much as possible while maintaining their sensorial attributes intact. Most of the methods to mitigate the formation of AA in food seek to inhibit the intensity of the Maillard reaction by reducing its precursors in raw materials and/or changing some process parameters. On the other hand, post-processing techniques which remove or trap AA after it is formed in foods may also be implemented. The use of several technologies combined to mitigate AA has been found to be the most effective approach, since the organoleptic quality of foods is not affected.[101] Conversely, suitable mitigation strategies for products such as coffee are still a pending issue.[102] In this section some of the most relevant AA mitigation technologies tested in potato- and cereal-derived products as well as coffee are discussed.

Reduction of precursor levels in raw materials

Asparaginase pre-treatments have been suggested as a promising technological intervention for AA mitigation. Asparaginase is claimed to significantly reduce AA levels by converting asparagine into aspartic acid, maintaining intact the sensorial attributes of the final product.[103] However, incorporation of the enzyme into food product seems to be the key factor in terms of AA mitigation efficiency.[104]

Some trials have been undertaken to study the use of asparaginase in potato systems. It is evident that the delivery of an enzyme into the potato tissue is a challenging task. However, immersing potato strips and slices in an asparaginase solution has shown an AA reduction of 30% (from an initial AA content of 2075 μg kg^{-1}) and 15% (from an initial AA content of 2047 μg kg^{-1}) in French fries and potato crisps, respectively.[105,106]

Currently, the AA reduction options available are applicable to a number of cereal products; however, some are product specific.[107] Fermentation is a tool to lower the occurrence of the precursor asparagine in cereal products. Hence fermented bread or crisp bread have lower AA content compared to bread that is leavened by baking agents. While yeast fermentation may assimilate up to 80% of the asparagine in a dough, sourdough fermentation of ryebread showed a more modest asparagine assimilation of 17%.[108] Applying longer yeast fermentation may reduce AA.[109] For other cereal products, substituting the ammonium carbonate leavening agent with sodium carbonate, substituting fructose with sucrose and adding antioxidants may also reduce AA.[110]

Another promising technical solution is the use of asparaginase.[111] This enzyme has the potential to achieve a 60–90% reduction (from an initial AA content of 1400 μg kg^{-1}) for some fried pastry products.[112]

Additionally, some studies performed in biscuits have shown a significant influence of the matrix composition on the capacity of asparaginase to reduce AA. For these baked products, high water content, which favors precursor mobility, promoted the enzyme capability of AA mitigation in the final product (48% and 58% of AA reduction were achieved in formulations with 10% and 20 % of water added).

Conversely, the presence of fat significantly reduced the enzyme activity (69%, 62% and 58% AA reduction corresponding to 0%, 8% and 15% fat concentration) as compared with the fat-free formulation. Regarding this last phenomenon, the authors hypothesized that the presence of fat would hamper the interaction between the precursors in the aqueous phase, leading to lower amounts of AA.[113] Previous results may confirm that any condition which favors substrate diffusion and its contact with the enzyme could lead to a greater reduction in AA levels. For instance, for fried potatoes, different authors have shown that the asparaginase mitigation effect is improved when the raw material is blanched before enzyme application.[105,106,114,115] Besides that, the sugar concentration of raw material is considered a determining factor for AA formation in potato products. [116] Thus an alternative possibility to mitigate AA in these products is to reduce their sugar content by blanching before thermal processing.[117] Many studies find a reduction of AA formation in potato products by changing the blanching conditions.[118] Regarding this issue, blanching temperature may be considered a critical factor of this pre-treatment, since during blanching some changes in the microstructure of raw potatoes are produced. These changes may not only affect the desirable texture of the final product but also the diffusion of precursors, provoking a diminishment in their extraction, and subsequently higher AA formation during frying.[119] Several authors have concluded that the application of low temperature and long blanching time (∼70 °C, 15 min) may be considered an effective AA mitigation technique for fried potatoes, since under these pre-treatment conditions not only is AA reduction achieved but also some desirable quality attributes such as firmness and low oil uptake are maintained.[114,117,118,120]

Changes in process parameters

AA formation in foods may be influenced by several factors during their processing, such as temperature, pH and addition/immersion of amino acids and salts.[48]

Considering that fried potatoes represent the major contributor to AA intake, several authors have studied the effect of frying conditions on AA formation. Results have shown that an alternative to mitigate the precursors of AA formation may be to decrease the frying temperature. In this sense vacuum frying seems to be a possible technology to reduce the AA content of fried potatoes. For instance AA has been reduced by about 90% (from an initial amount of 1900 μg kg^{-1}) in potato crisps fried under vacuum conditions.[121]

Interestingly, some authors have developed AA mitigation technologies based on lowering the pH of food products. Rydberg *et al.*[122] studied the effect of pH on AA formation, concluding that the dependence of AA formation exhibited a maximum around at a pH value of 8. Lower pH values enhanced AA elimination and decelerated its formation. The beneficial effect of low pH results primarily from protonation of the reactive free R&bond;NH_2 group of asparagine to the non-reactive R&bond;NH_3^+ form.[123] AA reductions were obtained in potato crisps by immersing in acetic acid solution (90%) or soaking or blanching in citric acid solution (50%).[124] However, low pH may adversely affect the taste of foods according to the organic acid concentration.[125]

Likewise, the addition of free amino acids other than asparagine or from a protein-rich food component to a model or food matrix is reported to strongly reduce the AA content, probably by promoting competing reactions and/or by covalent binding the AA formed.[126]

Among other amino acid compounds adding the amino acid glycine was found to reduce the AA formation in cereal- and potato-based products (reductions level between 30% and 70% compared to the control).[52,124,127]

However, this 'glycine methodology' still needs some improvements due to its negative impact on the flavor profile of the final products.[128] The addition of glycine to potato-based model systems heated at 180 °C for 5–10 min has been shown to significantly alter the distribution pattern of alkylpyrazines,[128,129] which are important flavor volatiles in baked potatoes.[130] It could be explained since the Strecker degradation of glycine leads to formaldehyde, which may react directly with dihydropyrazine intermediates to give alkylpyrazines with an additional methyl substituent derived from glycine.[128,129]

Additionally, NaCl proved to have considerable inhibitory effects on the AA formation in an equimolar mixture of asparagine/D-glucose (reductions of ∼40% in comparison to a mixture without NaCl). The effective lowering of the AA content was observed already at 1% NaCl addition, which makes it applicable to real technological procedures of food processing.[131] In fact, the dipping of potatoes in salt solutions of NaCl[132,133] or $CaCl_2$ [134] prior to frying has also shown a protective effect against AA formation in fried potatoes. Pedreschi *et al.*[132] observed AA reductions of 62% in potato slices blanched in NaCl solutions; however, almost half of this percentage (∼27%) could be attributed to the effect of NaCl and 35% to the effect of the slight heat treatment during the salt immersion step (25 °C for 5 min). These authors suggested that the addition of salt in the water solutions changes the osmotic potential and, in order to establish equilibrium, the liquid is transported out of the potato and a simultaneous washing out of reducing sugars occurs. Regarding calcium salts, these have been utilized only in low concentrations to avoid an off-taste.[134] For this application a higher AA reduction was obtained (about 95% compared to untreated control), probably due to ionic and electronic association between the $CaCl_2$ and asparagine which suppresses early-stage Maillard reactions. However, Ca^{2+} tends to influence pectin binding (possibly cross-links) in the cell walls, and harder and more brittle crisps are obtained.

It is worth noting that some of these AA mitigation strategies are not only associated with a loss of quality attributes but also

with an increase in the formation of other toxic compounds. For instance, the use of Ca^{2+} and glycine is reported along with a concomitant effect on hydroxymethylfurfural (HMF) concentration.[102] In the first case, this may be explained by cations preventing the formation of the Schiff base, changing the reaction path towards the dehydration of glucose and leading to HMF.[135] On the other hand, when glycine is added, overall Maillard reaction is accelerated, improving HMF generation.[136]

Post-processing techniques which remove or trap AA formed

Removing or trapping AA after it is formed with the aid of chromatography, evaporation, polymerization or reactions with other food ingredients has also been applied to food products such as coffee.[137]

Some authors have found that increasing the degree of roasting of coffee resulted both in a desirable decrease in AA levels and in an improved radical-scavenging capacity by concurrently formed melanoidin antioxidants. It is hypothesized that nucleophilic amino groups of amino acids from the proteinaceous backbone of melanoidins react via the Michael addition with AA, although the exact mechanism is unknown.[138] It is suggested that addition of soluble melanoidin could modulate the content of AA in the final coffee brew.[57]

Similarly, the removal of AA from coffee through supercritical CO_2 extraction has been investigated. Supercritical treatment may reduce AA content by up to 79% (with the addition of ethanol like supercritical fluid) without affecting the caffeine content of the coffee. However, to consider this method as a mitigation technology it is necessary to test its influence over the sensorial quality of the final product.[139]

Finally, the use of bacterial enzymes to control AA levels in coffee has been studied. Although AA was not totally degraded at higher concentrations of coffee in water, it totally disappeared in a 10 mg coffee mL^{-1} solution in water, which is a coffee concentration slightly more diluted than what people commonly drink.[140]

CONCLUSIONS

- The natural content of reducing sugars and amino acids in raw materials, food matrix microstructure and processing conditions has a strong influence on AA formation during heating.
- AA can be significantly reduced by reducing the heat load (time and temperature) during heat processing.
- AA can in most cases be reduced but not totally removed.
- AA mitigation techniques are based principally on removing AA precursors from raw materials and/or diminishing the intensity of the factors which favor Maillard reaction.
- The feasibility of many AA mitigation techniques on an industrial scale and their effects on the overall quality of heat-processed products have not been evaluated in most cases.
- AA mitigation in some food matrixes such as coffee and the effect of combining different AA mitigation techniques should be further investigated.
- Food safety authorities have adopted the view that AA is an undesirable, though apparently inevitable, consequence of baking, frying or roasting starchy foods.
- AA is considered a human risk at concentrations found in food, based on repeated 2-year cancer studies in rats and mice.
- Current policy is that practical measures should be taken voluntarily to reduce AA formation in vulnerable foods and so lessen human exposure to this chemical.

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