Acrylamide in widely consumed foods

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Acrylamide (AA) is considered genotoxic, neurotoxic and a “probable human carcinogen”. It is included in group 2A of the International Agency for Research on Cancer (IARC). The formation of AA occurs when starch-based foods are subjected to temperatures higher than 120 °C in an atmosphere with very low water content. The aim of this literature work is to shed light on the toxicological aspects of AA, showing its regulatory evolution, and describing the most interesting mitigation techniques for each food category involved, with a focus on compliance with EU legislation in the various classes of consumer products of

industrial origin in Europe.

Keywords: acrylamide; reducing sugars, asparagine, French fries; glycation reaction; Maillard reaction;

# Introduction

Acrylamide (AA) is a process contaminant that forms during the transformation and cooking of some foods. It can be formed through a glycation reaction (commonly called ‘Maillard reaction’), which is a particularly important reaction that also leads to the formation of melanoidins, which give foods the flavours and colours that we know (Mottram et al. 2002; Stadler et al. 2002).

The formation of AA occurs when starch-based foods are subjected to temperatures higher than 120 °C and at low moisture (Robert et al., 2004). Under these conditions, the reducing sugars (e.g., glucose, fructose) react with free asparagine (AS), with the formation of AA as a contaminant (Zyzak et al., 2003). This results in significant concentrations of AA in a number of widely consumed foods, such as, in particular, fried potatoes (i.e., chips/‘French fries’, crisps) (Masson et al., 2007), bread (Rufian-Henares et al., 2007), baked foods (e.g., biscuits, crackers, crispbreads) (Gokmen, 2014), coffee (Schouten et al., 2020), various snacks and baby food (Esposito et al., 2021).

Following data obtained with animal models, in 1994 the AA monomer was classified as a probable human carcinogen (group 2A) by the IARC (1994). This related to its use in specific production cycles, essentially in industrial chemistry (Pennisi et al., 2013). However, in 2002, the main source of exposure to AA for the general population was discovered in some widely consumed foods (Lofstedt 2003; SCF 2002; Tareke et al. 2002). From this point on, there were many studies that were designed to identify both the pathways of formation of this AA and the potential problems for humans following its daily intake (Duarte-Salles et al., 2013; Kadawathagedara et al., 2018a and 2018b; Wang et al., 2020; Zha et al., 2021). The problem thus mainly concerns AA formation during specific processes in the food industry, although this is also relevant to catering and home cooking. Indeed, foods cooked in the home are extremely heterogeneous, because this is also linked to the different cultures and traditions of the various populations around the world (Mihai et al., 2021; Mesias et al., 2020a; González-Mulero at al., 2021). Over the years, the European Commission has requested that the Member States and the EFSA collect data on AA toxicity and the levels of AA in foods.

From the latest EFSA reports, it has emerged that many animal studies have confirmed that AA can cause neurological damage, along with cytotoxic and genotoxic damage that can result in the development of cancers (EFSA, 2015). Furthermore, following a series of recommendations by the European Union (EU) following the monitoring of AA levels in food, a new EU Regulation was issued in 2017: N° 2158/2017. This EU Regulation defined the maximum reference levels for AA on the basis of what the food industry must obtain as the lowest possible contaminant levels, along with an indication of potential mitigation strategies.

The purpose of the present study was to take into consideration AA contaminations in a number of human foods, some of which we have reported on previously (Pantalone et al. 2021), and we are still working on. The present review also includes a careful evaluation of these foods and of their safety with the definite aim to propose some effective mitigation techniques on the AA formation

# Overview on acrylamide

## General information and chemical-physical properties

Chemically, AA is also known as 2-propenamide (*i.e.*, CH2=CH-CO-NH2), and as such it is an α,β-unsaturated organic carbonyl compound. It is highly soluble in water, and has the form of a white and odourless crystalline solid (EPA, 2010).

The chemical-physical characteristics of AA are given in Table 1

(Table 1 should be inserted here)

This molecular structure of AA defines a polar amide group and a vinyl functional group that allows its polymerization (Fig. 1) (Matoso et al., 2019). AA polymers, which are not considered toxic (Raffan and Halford, 2019), form quickly at the melting temperature or under exposure to UV irradiation (EPA, 2010). They are used in many industrial processes, such as flocculants in drinking water, preparation of polyacrylamide electrophoretic gels, production of dams and as a sealant in the construction industry (Herth et al., 2015).

(Figure 1 should be inserted here)

***Brief history and first evidences of its toxicity***

As AA has been used in industry since the 1950s, the initial toxicity studies focused exclusively on occupational exposure and cigarette smoke, through inhalation and skin absorption (Sorgel et al., 2002). A very important case was an accident that occurred during construction of a railway tunnel in Hallandsas (Sweden), where the grouting agent used contained AA and N-methylolacrylamide. This then leached into the waters and contaminated the surrounding environment.

The exposure of workers to AA was demonstrated to be through the formation of haemoglobin adducts (as exposure biomarkers), with irritation of the eyes, skin and respiratory tract, and the consequent neurotoxic effects (Hagmar et al., 2001). However, in one study, Bergmark (1997) also noted the presence of these adducts in individuals who were non-smokers and who had not been exposed professionally to AA, indicating that the source of this contamination was unknown at the time.

Then in 2002, researchers from the Swedish National Food Administration and the University of Stockholm raised the alarm, indicating the presence of AA in foods (Lofstedt, 2003). This thus revealed what can be considered as the main source of exposure for the entire population. The Swedish research team of Tareke et al. (2000) had already identified carcinogenesis markers in rodents fed with fried foods. They then identified high levels of AA in starch-based foods that had undergone very intense heat treatments, with the highest value reported as 1739 µg/kg in fried potatoes (Tareke et al., 2002).

***Acrylamide as a process contaminant***

Through these initial discoveries, and along with many subsequent studies, we can affirm today that AA is a process contaminant. It is a substance that is harmful to health but is not initially contained in the food matrix; instead, it can be generated during food processing that involves heat treatments such as frying, roasting and baking (Curtis et al., 2013; Halford et al., 2012). AA is thus formed starting from compounds already available in the foods in which it develops, such as, in particular, fried products based on potatoes (i.e., chips, ‘French fries’, potato crisps), baked goods (e.g., biscuits, crackers, crispbreads), roasted coffee, various snacks, and some baby foods. Since these are foods that are produced on a large scale, this does not only involves their preparation in the home, but also their catering and industrial production (Gokmen, 2015).

***Main effects on humans***

The potential effects of AA on humans are associated with its epoxide metabolite glycidamide, as this can interact with macromolecules, proteins and nitrogenous bases, to cause neurotoxicity (Friedman, 2003), genotoxicity, carcinogenesis, embryotoxicity and a potential endocrine disruptor (EFSA, 2015).

Over the years, various organisations have set themselves the goal of defining the mechanisms of formation of AA in foods, and the possible effects on humans with respect to their daily consumption. In line with what was stated by previous studies, such as those of the Joint Food and Agriculture Organisation (FAO)/ World Health Organisation (WHO) Expert Committee on Food Additives (JECFA, 2011; JECFA, 2005), in June 2015, the EFSA Scientific Panel on Contaminants in the Food Chain (EFSA CONTAM, 2015) published the latest scientific opinion on risk assessment for the population. From the data collected, it emerged that AA is a potential carcinogen for all age groups. Infants and children are more exposed to AA, with levels of 0.5-1.9 µg/kg body weight reported, while adolescents, adults and the elderly have mean levels of 0.4-0.9 µg/kg body weight. To date, the debate on the risks of dietary exposure to AA remains open, as often the effects detected in animal studies have been the result of the use of high doses that do not specifically correspond to estimates of actual daily consumption (EFSA, 2015). However, considering not just AA itself, but also its genotoxic and carcinogenic metabolite glycidamide, it shold be noted that at any level of exposure to a genotoxic substance has the potential to damage DNA and cause cancers. Therefore, it is not really possible to establish a "tolerable daily intake" (TDI).

In light of this, the dose range has been estimated within which AA is likely to result in slight, but measurable, incidence of tumours or other potential effects that have been described previously. This is based on the ‘benchmark dose’ (BMD), where the lower limit of this interval for an extra risk of 10% is the lower BMD confidence interval, or BMDL10. For tumours, the EFSA has defined a BMDL10 of 0.17 mg/kg body weight/day, while for the further toxic effects of AA, a BMDL10 of 0.43 mg/kg body weight/day has been defined.

For substances that are considered carcinogenic and genotoxic, the EFSA uses the concept of a margin of exposure (MOE) approach; i.e., the ratio between the dose at which a small but measurable adverse effect is observed the first time, and the level of exposure to the substance in question. On this basis, a food with a MOE of 10,000 can be considered harmless to public health. However, the MOE for AA is a lot lower; indeed, for the average exposures estimated, this has been reported as from 89 to 425, while for maximum exposure levels, from 50 to 283. These latter values are especially relevant for children, who are heavy consumers of foods that contain AA. This is of particular concern due to the neoplastic effects that can occur (EFSA, 2015).

As AA is an unavoidable contaminant, there was the need to issue a new EU Regulation more recently, as N° 2158/2017, which entered into practice from 11 April, 2018. This regulation concerns all operators in the food sector, and it also establishes mitigation measures. To reduce AA quantities, the reference levels in foods are defined as being ‘as low as reasonably achievable’ (ALARA). Then in 2019, the ‘FooddrinkEurope’ groups that represents the European food and drink industry provided their guidelines, along with different strategies for mitigation of AA levels in finished food products, starting from the agronomic choices, and covering recipes and processing techniques (FooddrinkEurope, 2019).

The attention of the food industry towards more conscious production choices must also be associated with better knowledge of the health risks on the part of the population. Indeed, assessment of the impact of AA on health is made difficult by the many variables that come into play, which include not just the choice of cooking methods, but also the increase in the consumption of processed (and ‘ultra-processed’) foods, which has reached some 25% to 60% of the total daily energy intake (Morales et al., 2020).

## Production and presence of acrylamide in foods

The main mechanism for the formation of AA in food recognized since the beginning of the 21st century (Mottram et al., 2002; Stadler et al., 2002; Friedman, 2005) is the reaction between reducing sugars (e.g., glucose, fructose) and amino acids (as mainly AS) at temperatures above 120 °C and at low moisture (Ubaoji and Orji, 2016). This process is commonly called "glycation reaction" and is also widely defined as the ‘Maillard reaction’, as the French chemist who described it, in 1912. It consists of a series of ‘non-enzymatic browning’ reactions that are responsible for the typical colour and aroma of cooked foods, but which can also produce unwanted compounds.

In 1953, the chemist John Hodge was the first to provide a detailed scheme of these reactions (Halford et al., 2010). The formation of AA occurs in the presence of free AS. As shown in Fig. 2, the glycation reaction involves an initial phase of glycosylation, in which the carbonyl group of the reducing sugar reacts with the amino group of the amino acid; this leads to the formation of a Schiff base, a glycosylamine. This is unstable, and so tends to rearrange itself, to produce compounds known as ‘Amadori compounds’ if the starting sugar is an aldose, or ‘Heyns-Carlson compounds’ if the starting sugar is a ketose (Wenhua and Xu, 2005).

(Figure 2 should be inserted here)

These reactions are influenced by pH and temperature, whereby at high temperatures, the Amadori compounds can enolyze into α-dicarbonyl compounds. These α-dicarbonyls can then react with amino acids, to result in oxidative deamination and decarboxylation. These, in turn, lead to the formation of aldehydes and Strecker α-aminoketones, along with release of CO2. In the last phase of the reactions, the Strecker aldehydes reacts with free AS, with the formation of AA. The Strecker degradation and the various intermediate reactions, which include the polymerisation of hydroxymethyl furfural, can give rise to melanoidins (i.e., brown pigments). These are responsible for the characteristic colour of the surface of cooked foods (Robert et al., 2004; Vinci et al., 2012; Yaylayan and Stadler, 2005). However, the complete information regarding the different pathways for the formation of AA has not been fully defined to date, mainly because food matrices are generally much more complex than model systems.

It is well documented that in a glucose/AS system, the Schiff base undergoes decarboxylation to generate N-(D-glucose-1-yl)-3-aminopropionamide. This intermediate can, in turn, undergo an Amadori rearrangement to produce N-(1-deoxy-D-fructose-1-yl)-3'-aminopropionamide. Both of these intermediates can result in the generation of AA, either directly or through the formation of free 3-aminopropionamide (Locas and Yaylayan, 2008).

Another possible pathway for the formation of AA in foods that are rich in lipids is from the unsaturated aldehyde acrolein. When the triglycerides in oils are subjected to prolonged and repeated high temperatures, such as during frying, they undergo hydrolysis, to release glycerol and fatty acids. Acrolein is produced from glycerol by dehydration, and it can then be converted to acrylic acid by oxidation. Acrylic acid, in turn, can generate AA through its condensation with the amino group of free amino acids in the food (Mestdagh et al., 2008a). However, acrolein usually reacts with other compounds, rather than undergoing oxidation to acrylic acid, so this pathway is of lesser importance for the formation of AA (Gokmen, 2015).

Proteins in foods also represent a possible pathway for the formation of AA during cooking. AA can form from alanine-containing peptides that are released during pyrolysis processes that involve wheat gluten (Claus et al., 2006).

The production of AA depends on many factors, which include the nature of the reactants, their concentrations, the temperature-to-time ratio, the pH of the medium, and the degree of moisture. Furthermore, under conditions of drying and heating over prolonged periods, foods rich in sugars and AS can promote the formation of AA even at temperatures below 100 °C, such as seen for plums (Becalski et al., 2011).

The concentrations of AA in the external areas of foods can also be up to 20 times higher than in the core (De Vleeschouwer et al., 2008). This arises because with the increase in temperature, more favourable conditions are created on the surface of the food for the production of AA, particularly as the moisture in this area is usually a lot lower than for the more internal parts (Sansano et al., 2017; Vinci et al., 2012; Brathen and Knutsen, 2005). This can also be relevant, as the external colour of many foods is one of the fundamental quality parameters for the acceptance of the product by consumers. Here, several studies have shown that there is a correspondence between the colour of the food and the amount of AA that is formed (Michalak et al., 2017; Mestdagh et al., 2008b). Indeed, AA is an integral part of the browning process, so the search for an optimal combination of temperature and final degree of browning can represent a good method for controlling the formation of this unwanted compound.

An EFSA report in 2015 included the levels of AA in foods that had been collected by the EU Member States and various food associations (EFSA, 2015). From the data in this document, it is possible to note some particularly important aspects. As shown in Fig. 3, the highest AA levels are found in vegetable chips, with a mean of 1846 μg/kg, and then in coffee substitutes, with a mean of 1499 μg/kg (for chicory-based substitutes, this can be even higher, at 2942 μg/kg).

(Figure 3 should be inserted here)

Data collected by the EFSA and subsequent cohort studies on consumer practice have generally been obtained through the administration of frequency questionnaires, food diaries and/or 24-h recall. These have shown that the main foods that contain AA that are consumed by the ‘Western’ populations in particular are highly processed foods that are based on potatoes, coffee, cereals and baked goods (EFSA, 2015; Kotemori et al., 2018). Asian (and other) populations, on the other hand, are less exposed to AA due to their different food use (Kotemori et al. 2018).

Starting from the consumption percentages reported in the EFSA "Scientific Opinion", it is possible to obtain an average of the estimated consumption of the different food categories, and to actually understand which foods are involved in the intake of AA. As explained in a review by Raffan and Halford (2019), and comparing the AA contents in foods (Fig. 3) with the relative food sources for AA in the diet in Fig. 4, it emerges that the AA intake is not so much due to high levels present in specific foods, but rather to the high amounts of certain foods that are consumed. Indeed, in Fig. 4 it is possible to note the primary role of potato products in the foods consumed, followed by bread; as can be seen from Fig. 3, although bread is at the lower end of the AA levels, it is actually in the foreground in the intake of AA because it is one of the most consumed foods in the world. Furthermore, a lot of bread is consumed toasted (i.e., with higher levels of AA), along with coffee (EFSA, 2015).

(Figure 4 should be inserted here)

As would be expected, the relative consumption, and therefore the contribution of the different food categories, will vary according to the age group considered. For infants, the main foods that contribute to AA exposure are "Processed baby foods, other than cereal based", "(non-fried) Potato-based foods" and "Processed cereal-based baby foods", which represent 60%, 48% and 30%, respectively, of their total dietary exposure to AA (EFSA, 2015).

For children and adolescents, the greatest contributions will be from "Fried potato products" (10%-51%), followed by "Soft bread", "Breakfast cereals", and "Biscuits, crackers, crispbreads" (EFSA, 2015). Then when we come to adults and the elderly, the dietary exposure to AA will be more heterogeneous. Here, the main food categories will be "Fried potato products", "Soft bread" and "Coffee", which represent 23% to 49% of the total mean exposure. These will then be followed by "Biscuits, crackers, crispbreads", "Cake and pastries" and "Breakfast cereals" (EFSA, 2015).

As already indicated, children are the most exposed group to AA, due to the higher food intake per kilogramme body weight (Mojska et al., 2012), and their tendency to take ever greater quantities of AA via their food. In the world of adolescents in particular, this will also be due to increased consumption of processed and ultra-processed foods (Morales et al., 2020).

## Stability of acrylamide during storage of foods

The large number of foods, with AA inside before storage, prompts us to be cautious on emanating any rule on its abatement. We retain that the priority is to specify the majors physical conditions to take into consideration: storage temperature, together with the water content and any conservation modality (*e.g.* in inert atmosphere rather than under vacuum etc.), represent the main parameters to take into consideration. Although AA is commonly considered stable, at least at temperature below its melting point (84.5 °C), where polymerization become dominant, other kind of transformations can be equally hypothesisable. For example some AA adducts can be generated during the storage of canned coffee. Among milk and black coffee, it seams that the former showed a major decrement (75%), at least at the temperature of 37 °C, while at lower values such 0 °C and room temperature, the effect is lower and only in favour of canned milk coffee. The transformation mechanism is not completely clear, even if some endogenous protein seems can react with AA forming stable adducts (Yoshioka et al. 2020). Similarly Michalak et al. (2016) analysed some foods with long shelf-life and observed that the ε-NH2 and SH groups present in the amino acids can interact with AA forming adducts that are considered not particularly dangerous for the consumer, and again the reaction rate seems to be dependent form the storage temperature (Hoenicke and Gatermann, 2005).

# Acrylamide toxicology

Many studies on mammals and *in-vitro* models have confirmed that following its ingestion in food, once AA is no longer covalently linked to the molecules of the food matrix, is can be rapidly absorbed in the gastrointestinal tract. This occurs through passive diffusion, and then it further diffuses to different tissues to thus be distributed throughout the body, with no evidence of its specific accumulation. The fate of AA in humans appears to be qualitatively similar to that in rodents (EFSA, 2015; Sansano et al., 2017). Of note, in a study by Schettgen et al. (2004) on volunteer mothers and newborns, it was shown that the free AA in the maternal blood can cross the placenta and the blood-milk barrier. This suggests the possibility for damage to the DNA of the developing child.

Once absorbed, AA is metabolised in the liver, where it undergoes phase I metabolism. Thus, the microsomal cytochrome P450 2E1 (CYP2E1) enzyme promotes the conversion of AA into the epoxide glycidamide (Fig. 5 - pathway (a)) (Kurebayashi and Ohno, 2006). Indeed, it is this glycidamide that is mainly responsible for the genotoxic effects of AA intake *in vivo*, because it can create adducts with DNA and proteins (Friedman, 2003; Katen, Roman, 2015). The high consumption of processed and ultra-processed foods that is seen particularly for adolescents can induce the enzymatic activity of CYP2E1 by increasing the formation of the reactive metabolite (Zhang Yiju et al., 2020).

(Figure 5 should be inserted here)

Subsequently AA and glycidamide can undergo a phase II detoxification reaction, with conjugation to glutathione via the enzyme glutathione S-transferase. This is an important detoxification mechanism that reduces the reactivity against cellular molecules. The AA and glycidamide will thus be in their free forms only for a minimal time, because they tend to undergo conjugation easily (Gokmen, 2015).

Once AA and glycidamide are conjugated with glutathione, they can undergo subsequent conversion to mercapturic acids, N-acetyl-S-(2-carbamoylethyl)-L-cysteine (AAMA), N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA) and N-acetyl-S-(1-carbamoyl-2-hydroxyethyl)-L-cysteine (iso-GAMA). They can then be excreted in these forms, mainly in the urine (Luo et al., 2015; Zhang et al., 2020). Glycidamide can also be spontaneously or enzymatically hydrolysed into glyceramide, although this is a less important detoxification pathway (Luo et al., 2015; Gokmen, 2015) (Fig. 6).

(Figure 6 should be inserted here)

Toxicokinetic studies allow us to better understand the toxicological profile and dietary exposure of AA. In a very recent study, Zhang et al. (2018) developed a UHPLC–tandem mass spectrometry method to simultaneously evaluate AA compounds and their metabolites in samples from rats and humans. In rats, the peak of formation of mercapturic acids was greater than the conversion of AA into glycidamide, and the most excreted compound was AAMA: AAMA **>** AAMA-sul (the S-oxide of AAMA) **>** AA > (GAMA) **>** iso-GAMA **>** glycidamide). In humans, out of a sample of 10 non-smoking students, the glycidamide levels were higher in males, while those of AAMA were higher in females (Zhang et al., 2020). Also, an analysis conducted by Wang et al. (2017) on a group of 110 Chinese students reported that urinary excretion of the various metabolites was affected by sex and body mass index (Fig. 7).

(Figure 7 should be inserted here)

It also needs be considered that during digestion, processes take place that can increase as well as counteract the toxic effects of AA. This will also depend on the basis of the type of starting matrix and the different Maillard compounds that are generated (Friedman, 2005; Sansano et al., 2017). Indeed, the gastric pH can promote the conversion of reaction intermediates into AA, and pepsin promotes the degradation of the food matrix, thus also promoting the release of AA from the food matrix (Hamzalioglu and Gokmen, 2015).

Sansano et al. (2017) investigated the kinetics of AA in the different digestive phases: oral, gastric and intestinal. This was evaluated in *in-vitro* models starting from different food matrices, which included fried potato products, coffee and its substitutes, crackers, biscuits, breakfast cereals and fried onion rings. Based on their intrinsic characteristics, different AA contents were noted for these foods. The foods that led to higher initial AA contents were those with the higher content of the precursors, as reducing sugars and AS, and with less moisture. The AA contents were also linked to the foods that were subjected to intense temperature treatments, such as coffee and fried potato products. The AA levels were monitored at 0, 15 and 120 min after food ingestion, and the results showed that pH and pepsin have key roles in the bioavailability of the AA. At the intestinal level, during the digestion of some of the starting foods, the formation of short peptides and free amino acids (e.g., cysteine, lysine) promoted the lowering of the bioavailability of AA through increased adduct formation (Sansano et al., 2017).

Berger et al. (2011) studied the bioavailability and biological activity of AA in rats. They compared AA supplied in water, to rats fed with French fries obtained from raw potatoes or with dough of starch, gingerbread and rye wholemeal crispbread. As only free AA is absorbed, no difference was noted between the urinary metabolite levels of the fed rats, except for the rye crispbread group, where the metabolite levels were 20% lower.

The toxic effects of AA and glycidamide are related to their induction of oxidative stress and to their reacts with the amino (-NH2) and sulfhydryl (-SH) groups of biological macromolecules. This can cause damage at the cellular and genomic levels, which can, in turn, affect the nervous system and increase the chances of developing cancers. Although the double bond of AA can promote alkylation of DNA and proteins, glycidamide is actually more reactive. The epoxy form of glycidamide (Fig. 5) contains two electronegative CO bonds, and due to their nucleophilic addition reactions, these can react more easily with nucleophilic molecules (Gokmen, 2015; Zhang et al., 2018).

## Biomarkers of exposure

To determine the exposure to AA over the short term and long term, various biomarkers have been identified where their levels are directly proportional to the amount of AA ingested. Compared to dietary estimates (e.g., frequency questionnaires, 24-h recall), these can provide more reliable estimates of the amounts of AA and glycidamide in the body.

The main biomarkers used are the mercapturic acids and adducts to DNA and haemoglobin in the urine and serum. The mercapturic acids are urinary metabolites and they are used as exposure biomarkers that cover a maximum period of the previous 48 h. AAMA has a half-life of 17 h, and GAMA of 25 h (Fuhr et al., 2006; Sams et al., 2015). These are thus relatively stable compounds that can be identified with high specificity and sensitivity (EFSA, 2015).

DNA adducts are markers of genotoxicity, as for N7-glycidamide-guanine in particular (see section 2.5). These are used to estimate the exposure to glycidamide in humans and other animals. As these are also removed over the short term (i.e., 2.6-7.4 days), they can indicate at most the exposure for the previous week (EFSA, 2015). Therefore, the metabolic periods of mercapturic acids and glycidamide adducts to DNA do not reflect consistent analysis of the cumulative exposure (Zhang et al., 2020).

Haemoglobin adducts are used to assess chronic exposure to AA. These are formed when AA and glycidamide create adducts with the N-terminal valine of the haem protein. The main haemoglobin adducts are N-2 carbamoylethylvaline and N-(R, S)-2-hydroxy-2-carbamoylethylvaline (Zhan et al., 2018). Once formed, these adducts are not repaired, and so they continue to accumulate over the life-time of the erythrocyte, thus reflecting the exposure to AA over the previous 4 months.

However, none of these biomarkers provide useful information as to the source of the exposure (Gokmen, 2015; Vesper, 2007).

## Acrylamide and oxidative stress

Oxidative stress can be defined as a condition in which the formation of reactive oxygen species (ROS) is not in tune with their neutralisation according to the body’s physiology (Rhaman et al., 2012). From the chemical point of view, ROS are highly reactive molecules that tend to damage the various cellular components. They can result in breakage of the double strand of DNA, and they can also damage proteins and polyunsaturated fatty acids of membrane phospholipids. To counteract the propagation of radical reactions due to the formation of ROS, the body can activate a series of enzymatic systems that include superoxide dismutase, catalase and glutathione peroxidase, along with non-enzymatic systems such as the production of antioxidant molecules (Arienti, 2016).

The toxicity of AA is thus associated with its induction of intracellular oxidative stress through this increase in ROS, which involve reactions with nucleophilic compounds and lipid peroxidation (Kopanska et al., 2015). Free radicals can attack the nitrogenous bases of DNA, to form various adducts, and also the membrane lipids, to promote the formation of malondialdehydes, which are highly reactive three-carbon dialdehydes that can lead to genome instability and cancers. In this way, AA can contribute to changes in gene expression and cell proliferation and differentiation, and in some cases, to the promotion of cell apoptosis (Lin et al., 2020; Kumar et al., 2018).

In mice treated with AA (0.5 µg/kg-40 mg/kg), there was a reduction in the availability of glutathione, as it was engaged in the detoxification of AA, and thus this did not allow the control of peroxides (Yousef and El-Demerdash, 2006). Under physiological conditions, these peroxides are reduced by the reduced glutathione/ oxidised glutathione system. Such a reduction in the availability of glutathione can result is blockage of the body’s defence mechanisms, to cause increased oxidative stress. In addition, in this study, Yousef and El-Demerdash (2006) reported increases in superoxide dismutase levels in plasma, testes, kidneys and brain; this favours the increase in superoxide anion levels. Also in a study conducted on adult rats with administration of 25 mg/kg AA for 21 days, Ansar et al. (2016) noted liver damage induced by oxidative stress.

Of note, high doses of AA have often been used in animal studies compared to the actual human daily AA consumption, and so continuing with human studies is extremely important to be able to fully define the possibility of a relationship between AA and oxidative stress. Indeed, in a cohort of 3270 adults, Wang et al. (2020) recently reported a direct correlation between the concentrations of urinary mercapturic acids and the presence of markers of oxidative damage, such as the 8-hydroxydeoxyguanosine DNA adduct, which is mediated by the hydroxyl radical, and 8-iso-prostaglandin F2a, which is produced by lipid peroxidation (Wang et al., 2020).

## Neurotoxicity

The only well-documented effects of AA on humans are related to its neurotoxicity (Hagmar, 2001, Friedman, 2003). Neurotoxicity refers primarily to occupational exposure, where workers come into contact with high doses of AA by inhalation and through skin adsorption. This can result in skin rashes, ataxia, peripheral neuropathy with axonial degeneration, numbness and tingling of the upper and lower limbs, and weakness of the skeletal muscles (Pennisi, 2013). There is a clear correlation between high levels of AA adducts with haemoglobin and the appearance of symptoms of the peripheral nervous system (Kopanska et al., 2018).

For diet-related exposure to AA, adverse neurological effects are less likely, although morphological changes to the nerves due to high AA consumption cannot be excluded (JECFA, 2005). The underlying mechanisms here are not yet fully known. It is generally accepted that the effects of neuronal toxicity are related to oxidative stress and the creation of adducts with neuronal proteins by AA binding to cysteine residues of the presynaptic membranes (Lo Pachin and Gavin, 2012). Indeed, AA can act directly on the nerve endings to cause synaptic dysfunction (thus blocking the flow of nerve impulses) and neuronal damage (Matoso et al., 2019). Furthermore, these toxicity mechanisms involve terminal degeneration of the central nerves, which has detrimental effects on the cerebral cortex, thalamus and hippocampus (Liu et al., 2017).

Many studies conducted on animals have shown symptoms that are similar to those following exposure in humans, which have been produced by high acute doses or lower doses over longer periods (Liu et al., 2017). For example, the exposure of rodents to repeated doses of 10 mg/kg to 50 mg/kg caused neuropathy, with an LD50 estimated at 100 mg/kg to 200 mg/kg (WHO, 2010). The degeneration of peripheral nerves and axons in the brain areas for memory, learning and cognitive functions was documented in another study (Matoso et al., 2019).

In 2020, Bu et al. (2020) used a three-dimensional neuronal model to show that AA represses neuronal differentiation, induces apoptosis, and promotes Tau protein hyperphosphorylation, which results in neurodegeneration. Neuronal degeneration is strongly linked to oxidative stress, and this mainly affects areas of the body with intense metabolic activity and cellular barriers, such as the nervous system.

Indeed, increased levels of malondialdehydes, which are the terminal products of the radical reactions of lipid peroxides that are responsible for cross-linking between different protein molecules, have been observed in different areas of the brain of rats exposed to AA (Kopanska et al., 2015; Arienti, 2016). Increases in malondialdehyde levels have also been noted in the erythrocytes, blood and neurofibrillary tangles of the brain of patients with Alzheimer's disease (Semla et al., 2017; Matveychuk et al., 2011).

Also for neurotoxicity, many studies are lacking in humans regarding direct correlations with AA intake in food. Liu et al. (2020) noted slight cognitive decline in elderly non-smokers who were exposed to very low daily doses of AA (0.24-0.27 µg/day). Thus, to date, it cannot be said with certainty that dietary exposure to AA causes neurodegeneration; however, AA appears to be at least a contributing cause to the various genetic, environmental and dietary factors that contribute to neuronal damage.

## Genotoxicity, cytotoxicity and carcinogenesis

As indicated, AA and its biotransformation product glycidamide are considered to be genotoxic, cytotoxic and carcinogenic agents. This arises from the cell damage that they can cause through induction of oxidative stress and formation of adducts with DNA bases.

To better understand the mechanisms involved at the cellular level, Nowak et al. (2020) exposed the Caco-2 cell line to increasing doses of AA over 24 h. This resulted in the induction of genotoxic and cytotoxic effects, with polarisation damage to the mitochondrial membrane, cell apoptosis, and oxidative damage to DNA, as a result of ROS. The greatest damage occurred using the highest AA concentration of 12.5 mM (Nowak et al., 2020).

Cytotoxicity was evaluated by Kacar et al. (2018) on A549 lung adenocarcinoma cells that were incubated for 24 h with AA from 0.5 mM to 20 mM. Using flow cytometry techniques and microscopy analysis, they showed that AA lowered cell viability (IC50, 4.6 mM), and the cells underwent nuclear condensation and fragmentation, break-up of the cytoskeleton, and membrane blebbing, which are typical characteristics of cell apoptosis (Kacar et al., 2018).

Although AA can form adducts with DNA, its direct effects on the genome appear to be mediated above all by glycidamide. Glycidamide has a greater propensity to covalently bind to the nucleophilic centres of DNA, to induce mutations and cell transformation. In particular, as indicated briefly above, glycidamide binds to the nitrogen in position 7 of guanine (Fig. 5 - pathway (b)) (Eisenbrand, 2020; Watzek et al., 2012). This particular adduct has great pro-mutagenic properties due to the formation of G-T transversions during DNA replication (Sempla et al., 2017; Besaratinia and Pfeifer, 2004).

The predominant role of glycidamide in binding to nitrogenous bases has been confirmed by studies on mice, in which those lacking CYP 2E1 and treated with AA showed lower levels of DNA adducts and signs of mutagenesis, compared to the control wild-type (Beland et al., 2015). Furthermore, later experiments conducted on male and female F344 rats given 5-10 mg/kg/day AA through their drinking water for 2 months indeed showed that AA is mutagenic through its conversion into glycidamide (Mei, 2010). Also, exposure of rodents to a single dose of 10, 20 or 30 mg/kg or repeated doses of 10 mg/kg for 1 or 2 weeks confirmed the role of glycidamide in the relevant increases in chromosomal aberrations and in micronuclei in bone-marrow cells (Alzahrani, 2011).

In a recent study by Holzl-Armstrong et al. (2020), knock-in mouse embryo fibroblasts for the human TP53 gene were isolated and treated with AA and glycidamide to determine whether these compounds can actually cause mutations. Their results showed mutations in specific codons that are typical of human cancers, such as those of the breast, ovary, colorectum and lung; furthermore, the mutant frequency was 9% in glycidamide cultures (1.1 mM for 24 h) compared to 0% in AA cultures (1.5-3.0 mM for 48 h) (Holzl-Armstrong et al. 2020).

Several studies have been carried out on B6C3F1 mice and Wistar and F344 rats, which were given water that contained increasing levels of AA for 2 years. In these rodents, the high AA treatments in particular promoted tumour growth in different tissues, such as in thyroid follicular cells in both sexes, and the formation of mammary fibroadenomas, Harder's gland adenomas, and skin, ovarian, oral cavity, stomach and lung cancers (Beland et al., 2015; Maronpot, 2015; EFSA, 2015).

As reported by the CONTAM panel in 2015, animal studies also show that AA is a potential carcinogen (EFSA, 2015); however, the available human data are limited and inconsistent, which is why AA remains a ‘probable human carcinogen’. Most epidemiological studies have included investigations of endocrine tumours, as strong associations have been seen between AA intake and tumour development in animals for the ‘hormone-sensitive’ sites, such as the mammary glands and the uterus (Adani et al., 2020; Hogervorst et al., 2019; Wilson et al., 2010; Olesen, 2008). Furthermore, AA has been associated with alterations in hormone levels in pre-menopausal and post-menopausal women (Adani et al., 2020).

In an epidemiological study of Danish post-menopausal women with a mean age of 57 years, Olesen et al. (2008) found a possible association between dietary intake of AA and breast cancer though an analysis of their levels of haemoglobin adducts. Then more recently, Obon-Santacana et al. (2017) reported that in non-smoking post-menopausal women, higher body mass index and higher alcohol consumption also resulted in increases in haemoglobin adduct concentrations (Obon-Santacana, 2017).

A systematic review by Adani et al. (2020) of non-smoking women showed that they have higher risk of developing endometrial and ovarian cancers associated with the consumption of higher doses of AA. On the other hand, there was no general association with breast cancer, except for pre-menopausal women with AA intake >20 µg/day.

Possible associations with consumption of higher doses of A have also been found for melanoma, multiple myeloma (10 µg AA: hazard ratio, 1.14) and follicular lymphoma in men (10 µg AA: hazard ratio, 1.61) (Bongers et al., 2012; Lipunova et al., 2017). However, from meta-analyses carried out within the last year, it emerges that there is no clear evidence of carcinogenesis, as the relative risks found for different types of cancers did not reach significance (Atabati et al., 2020; Benisi-Kohansal et al., 2021).

However, estimating the risk of cancer in humans is made difficult by the many variables found during data collection, such as physical condition and predisposing genetic factors, different methods of cooking foods, exposure to various environmental factors, and social habits such as cigarette smoking and alcohol. Indeed, the risk of cancer is increased by the presence of AA not only in cigarette smoke (e.g., a single cigarette can contain 1.5 µg AA), but also in food packaging, cosmetic products, and contaminated water (Busova et al., 2020; Nowak et al., 2020). Furthermore, dietary frequency questionnaires also have numerous limitations, although they are a good method to ensure follow-up of subjects (Pelicioli Riboldi, 2014; Liu et al., 2019, Atabati, 2020). Further studies are therefore needed to better establish the role of AA in the development of cancers.

An important step in this research was taken by the group of Zhivagui, who traced a distinctive ‘mutational signature’ in the genome that is induced by glycidamide; this is also seen in a wide range of human cancers (IARC, 2019; Zhivagui et al., 2019).

## Effects on reproduction and development

It is now known that there are various chemicals that can disrupt the endocrine system (i.e., Endocrine-Disrupting Chemicals, EDCs) now present in the environment, and that these are associated with various endocrine abnormalities. EDCs are exogenous substances in air, water, food and other products that can alter the production, release, metabolism and/or excretion of hormones, or can even imitate hormone activities. AA has been shown to be an EDC (Matoso et al., 2019), whereby it can cause dysfunctions in development and reproduction, thus compromising various aspects of human health (WHO/UNEP, 2012).

From the conclusions of the CONTAM panel in 2015, it is known that AA can negatively affect the male reproductive system. Taking the various studies together, it has emerged that the intake of AA can cause testicular atrophy, spermatic abnormalities, degeneration of the epithelial cells of the seminiferous tubules, damage to spermatogenesis, reduced copulatory activity, and changes in hormone levels (EFSA, 2015; Kumar, 2018).

Some animal studies have also indicated that the actions of AA on the nervous system are the basis of reduced motor capacity of mice during mating (Kumar et al., 2018). Also in rats, where Alkarim et al. (2015) studied the cumulative effects of AA on males and females exposed to low doses (0.4 µg/g). They showed effects on testicular weight and seminal vesicles, as well as on the ovaries and uterus. The testes showed atrophy and separation of the seminiferous tubules, with interstitial oedema in the testicular parenchyma. In females, there were cystic ovarian changes and marked decreases in mature follicles. Post-implantation embryo loss was also seen, with small numbers of live pups after birth (Alkarim et al., 2015).

More recently, to further investigate the toxicity of AA in terms of the female reproductive system, Aldawood et al. (2020) subjected female Wistar albino rats to 14 days of different doses of AA (2.5, 10, 50 µg/kg/day). From their serological and histological analyses of the ovaries, AA reduced the ovarian weight and the concentrations of progesterone and estradiol, and increased the levels of apoptosis, especially for the higher doses (Aldawood et al., 2020).

For foetal development, pregnant rats were subjected to 25 mg/kg/day AA (Erdemli et al., 2018). Biochemical and histopathological analyses of the foetal brain tissue showed increased levels of malondialdehydes and reduced levels of brain-derived neurotrophic factor and the antioxidant systems. Other animal studies have also report evidence of small litter sizes, reduced bone mineralisation, lower live-birth rates, and interference with the expression of genes involved in embryonic development (Zhu et al., 2021; Yu et al., 2019).

The spread of AA in food, its ability to pass through the placenta (Shettgen et al., 2004), and these effects demonstrated on rodents were the basis for the various epidemiological studies that have been conducted on pregnant women, to highlight the aspects related to development of the foetus. For instance, the Norwegian Mother, Father and Child Cohort Study (MoBa; from *Norske Mor and barn-undersøkelsen*) included 50,651 pregnant women and compared their levels of AA intake. The data were obtained through food-frequency questionnaires and the levels of haemoglobin adducts in the blood, which were related to the birth weights of the newborns. From the results obtained, and also excluding the non-smoking mothers, an AA association with reduced foetal growth and the birth of ‘small for gestational age’ children was noted (Duarte-Salles et al., 2013). More recent cohort studies have also had the same results (Kadawathagedara et al., 2018a). Moreover, starting from the MoBa study, while there was seen to be an important weight recovery for these children in the post-natal period and in childhood, they were also shown later to be more overweight, with greater development of obesity (Kadawathagedara et al., 2018b).

The role of nutrition is essential for good health of the mother and baby. Although there is the need for other epidemiological studies, it has been shown that exposure to toxic agents such as AA during pregnancy can negatively affect the growth of the child and have repercussions on their adult life. It must also be emphasized that the father can also have a crucial role in the transmission of DNA damage to subsequent generations (Katen and Roman, 2015).

# European legislation on acrylamide

Following the discovery of AA in food as a chemical process contaminant, the EU Scientific Committee for Food (2002) published a ‘first opinion’ on the general knowledge on AA. In this, they recommended that the Member States strengthen the means to reduce the quantities of AA, and to share in the progress of the information and analyses. As emerged from the JECFA, the first EFSA statement of 2005 also revealed concern for human health, as AA was found to be carcinogenic and genotoxic on laboratory animals. They thus indicated that steps needed to be taken to reduce AA exposure, particularly as they believed that the MOE was set too low in terms of medium and strong consumers (JECFA, 2005; EFSA 2005).

As early as 2006, the European food industry ‘FooddrinkEurope’ group that was known at the time as the ‘*Confederation des industries agro-alimentaires de l'UE*’ (CIAA) defined their guidelines for mitigation of the problems of AA. They thus provided their first ‘toolbox’, which provided the tools to support companies in the control of the risk phases of AA formation (CIAA, 2006).

In Europe, data on the AA levels in foods have been collected since 2003 by the EU Joint Research Centres Institute for Reference Materials and Measurement (JRC-IRMM), with the collection also by EFSA from 2007. In particular at the time, in their initial survey (as required by the EU recommendations), EFSA examined the levels of AA for the period of 2007, and compared these to those defined by the JRC-IRMM in the period of 2003-2006. According to the EFSA conclusions here, there had been no real downward trend, except for particular products. However, it could not be said that this was due to the mitigation techniques implemented by the industries (EFSA, 2009).

Thus, the first recommendations of the European Community (i.e., N° 2007/331/EC) were to monitor the levels of AA in food, and in particular for the three-year period of 2007-2009, to transmit the data collected to EFSA. These data collected by the Member States referred to food products that were known to contain higher levels of AA, or in any case to contribute significantly to dietary exposure: potato chips (French fries), potato crisps, potato products for home preparation, bread, breakfast cereals, biscuits, roasted coffee, canned baby food, cereal-based baby food, and other products.

The European Commission then issued new recommendations in 2010 (N° 2010/307/EU), in which it recommended continuation of this monitoring. Also, with the acquisition of more data, in 2010 the EFSA published the monitoring of the 2008 AA levels. Compared with 2007, these were for the most part unchanged, although tending to lower values; however, for some categories the AA levels were higher, such as potato chips, instant coffee and coffee substitutes (EFSA, 2010).

At this point, in 2013, the EU issued new recommendations with the aim to provide better understanding of the reasons why greater amounts of AA develop in certain products compared to other products that even belonged to the same category. To achieve this aim, indicative levels of AA were established for the different foods. In the event that a company (i.e., a food business operator [FBO]) found higher AA levels in their product than the recommended limit, an analysis had to be made of the production and processing methods they used, with the results communicated to the competent authorities.

Based on the EFSA monitoring published in 2011 and 2012, the EU issued the new recommendations (N° 2013/647/EU), particularly as there was a need to review some of the indicative levels of AA in food that had been defined in 2011, which were deemed too high. Then in 2013, the European Commission asked EFSA to publish a ‘Scientific Opinion’ on the potential risks of AA to human health; this was published 2 years later. From these latest EFSA conclusions that included the evaluation of hundreds of scientific articles, it emerged that there could actually be a risk that AA is carcinogenic for humans, and the European Commission issued EU Regulation N° 2158/2017.

In monitoring AA levels in fried potato chips that was carried out from 2002 to 2016 in Europe (Fig. 3), Powers et al. (2017) observed a real decrease from mean levels of 763 ng/g in 2002 to mean levels of 358 ng/g in 2011. This showed that the mitigation techniques defined had produced effects up to 2011, although despite this, the maximum levels of AA in the foods analysed were always higher than those required by the recommendations. Also, in the following years, the AA levels remained essentially unchanged, until 2016 when an increase was seen instead (412 ng/g) (Powers et al., 2017). These data were also in line with the data collected by the EFSA from 2011 to 2015, where again the levels of AA at the 95th percentile (P95) were higher than the indicative levels (EFSA, 2015). It therefore became necessary for FBOs to make further efforts to lower the AA levels in their products.

The new regulation of 2017 was created with the aim of raising the level of protection of human health, which thus also provided FBOs with mitigation strategies for AA levels in foods, based on ‘Good Hygeine Practice’. This was accompanied by hazard analysis and the recognition and management of critical control points (i.e., the ‘HACCP’ principles). In this Regulation, as the formation of AA is inevitable, the levels of AA that must be observed in foods were no longer defined as ‘indicative’, but as ‘reference’; as the ‘benckmark’ levels (Table 2).

(Table 2 should be inserted here)

In the previous recommendations of 2013, the indicative levels of AA were not intended as safety limits, but as levels that defined that the food with higher doses had the requirement for more detailed analysis of the production and processing methods, to monitor the process phases where the AA might have been formed. In the new regulations, the reference levels as given in Table 2 now indeed represent the maximum thresholds, above which the FBOs will have to review their entire process, to obtain the lowest possible levels of AA in the finished product (Raffan and Halford, 2019). In this context, the monitoring for AA must not affect the organoleptic characteristics and the microbiological safety of the food.

In 2019, the European Commission published its latest recommendations as N° 2019/1888/EU, which refer to regulation 2158/2017 for the monitoring of AA levels in some foods that had not yet been fully taken into account. These further foods might also contribute significantly to the exposure of the population to AA (Table 3). As indicated by the recent recommendations, to increase the levels of protection of human health, the data collected will have to be submitted on 1 October each year.

(Table 3 should be inserted here)

# Mitigation strategies for acrylamide in human foods

European Union Regulation N° 2158/2017 lists the mitigation measures that FBOs need to follow to reduce the concentrations of AA in their finished products. In general, these concern the monitoring of the various parameters that can contribute to increases in AA through the entire production chain, which mainly include the precursors used, the temperature and cooking time, and the pH and moisture during cooking. In addition to relating to ready-to-eat foods, contamination with AA also involves processed foods that are intended for cooking at home. Thus, the regulations contain obligations that are addressed to FBOs in terms of the information that needs to be included on the label of the products, to be sure not to increase the levels of AA during home cooking.

In view of the publication of the new regulations, the European food industry updated the ‘toolbox’ in 2019, with new tools added that can be adopted by companies (FBOs, including for small and medium-sized enterprises) to control the formation of AA. With greater awareness of the industrial processes and the different parameters that must be monitored, the means have been identified to specifically aim at the different categories and subcategories of food (FoodrinkEurope, 2019).

Thus, with the publication of the Scientific Opinion on AA in 2015, the EFSA established that AA in food increases the risk of developing cancer in all age groups (EFSA, 2015). Then European Commission Regulation N° 2017/2158 established the recommended mitigation measures for the food industry, and the reference levels to reduce AA in foods, and consequently its harmful effects on the population.

Furthermore, the food industry has been very closely involved in these processes, and is interested in prospective studies that evaluate the evolution of the trends of AA levels in foods, which have indicated that the incidence has increased in recent years (Delgado-Andrade et al. 2020). In this sense, several research groups from different countries have started to assess the exposure of AA in the different food categories regulated in 2017 in their respective countries. In the following sections, the main foods involved in this food exposure to AA will be considered with reference to the techniques used by FBOs to control the levels of AA.

## Fried potatoes

The botanical name of the potato is *Solanum tuberosum* L., which is a vegetable root (or tuber) that belongs to the *Solanaceae* family. Together with cereals, it is a fundamental component of world nutrition, either consumed as it is, or destined for the food industry. Over the years, the demand for processed potato-based foods and snacks has continued to increase, which has, unfortunately, resulted in greater exposure of the population to AA (Liyanage et al., 2021). Therefore, the primary objective of the changed practices recommended is to reduce the formation of AA.

From a chemical point of view, potatoes do not actually contain AA, but they do contain the main precursors for formation of AA: reducing sugars (e.g., glucose, fructose) and AS. When these are exposed to high temperatures under low moisture, this promotes its formation. AS represents 0.2% to 4% of the dry weight of potatoes, and it represents one third of the total amino acids in the free form (Halford et al., 2012; FooddrinkEurope, 2019). The chemical composition of potatoes varies according to the cultivar, soil, agronomic practices adopted, ripening stage of the raw materials, and storage conditions. The management of these phases, therefore, defines the need to choose a raw material that has naturally low levels of reducing sugars and AS, together with the choice of cooking and food processing techniques and conditions to avoid the formation of AA in the finished product.

To support these considerations, studies by Muttucumaru et al. (2014, 2017) were carried out with various field trials in England with different potato varieties. They investigated the formation of AA both at harvest and during storage based on the concentrations in the potatoes of glucose, fructose, free AS and total amino acids, along with the role of fertilisers. They showed that each variety responds differently due to a genetic component. The formation of AA precursors in the tubers also depends on the pedoclimatic conditions; indeed, it was observed that in certain potato varieties, the AA levels changed when the cultivation site was changed. In addition, they noted that for some varieties, the storage conditions influenced the potential for AA formation due to changes in the reducing sugars and AS. However, the storage conditions have a less decisive role in the formation of AA than the reducing sugars (Muttucumaru et al., 2014, 2017). Not surprisingly, the concentration of this last is considered a good indicator of the potential for AA production in food (FooddrinkEurope, 2019). Indeed, a first strategy to control the formation of AA in the finished product is to use a raw material that is naturally low in reducing sugars and AS.

The effects of mitigation are closely linked to the potato genotype. Therefore, a fundamental objective is to manage the concentration of these precursors by improving the agronomic techniques and defining the varieties that are indicated for specific production conditions, to maintain the final levels of AA as low as possible. This can also limit the more onerous actions (for FBOs) of changes in the production processes (Liyanage et al., 2021).

The role of AS is decisive only in contexts that are characterised by low levels of reducing sugars, and in relation to the pool of total amino acids (Knight et al., 2021). To reduce the formation of AA by acting on the AS levels, mitigation tests were carried out using the enzyme asparaginase, which catalyses the hydrolysis of AS into aspartic acid and ammonia, without affecting the other aspects of the final product (Rifai and Saleh, 2020). Field trials of new potato varieties with a silenced AS synthase gene have made it possible to obtain potatoes with lower levels of AS (FooddrinkEurope, 2019). By silencing the gene involved in the synthesis of AS synthetase 1 (*Asn1*) the formation of AA in the final product can be reduced by 52% to 78% (Rifai and Saleh, 2020).

Fertilizers might also have a role in the formation of AA, as nitrogen and sulphur affect the concentration of the precursors, and particularly of the free amino acids (Muttucumaru et al., 2013; Sun et al., 2020). Another parameter to consider is the time of potato collection and storage. Potatoes must be ripe at harvest time; indeed, more immature potatoes have higher contents of the AA precursors. Furthermore, potatoes need to be stored at temperatures above 6 °C to prevent the phenomenon of softening that is induced by low temperatures. Here, the AA levels in fries made from potatoes stored at 8 °C can be reduced by a factor of 10 compared to those stored at 4 °C (FooddrinkEurope, 2019). Furthermore, climate also has an important role in the formation of the AA precursors: to obtain lower levels of AA in the finished products, the ideal cultivation temperature of potatoes is between 15 °C and 20 °C (Rifai and Saleh, 2020).

Experimental models of AA formation kinetics can also help FBOs to better control the production phases, with each adapted to the different processing purposes. In the food industries, potatoes are generally subjected to frying in vegetable oil, both for the production of ‘ready to eat’ foods and as a pre-treatment for frozen products.

Palazoglu and Gokmen (2008) carried out experiments on potato samples, including French fries, and highlighted the linear relationships between sugar content, temperature and the formation of AA. During the frying phase, the heat from the hot oil increases the internal energy of the potatoes until their surface reaches the boiling point of water (103 °C). At this point the moisture is expelled in the form of steam. When the temperature exceeds 120 °C, there is AA accumulation along with increased reduction of the sugars. Furthermore, they observed that with frying models at 170 °C for different times (3, 4, 5, 6 min), the AA content was higher in the thinnest pieces of potato (Palazoglu and Gokmen, 2008).

In a more recent study of the kinetics of AA formation, Knight et al. (2021) used potato models where they altered the chemical composition with the addition of sugars and amino acids. In samples with different levels of glucose and fructose subjected to 155 °C, they reported that the formation of AA began after 80 s to 95 s of the frying, with the moisture was <6% (the critical point of dehydration). From this moment on, the production of AA occurred at a constant rate, whereby its formation was proportional to the initial concentration of the reducing sugars. Furthermore, the determining role of the low final moisture was highlighted. In this case, thin slices of potatoes were considered, as so-called potato crisps, in which the formation of AA occurred with constant speed with a uniform distribution over the entire surface (Knight et al, 2021).

Therefore, the main parameters of the production processes that influence the formation of AA, and which therefore must be monitored, are the temperature and time of the heat treatment, the heat transfer, the speed of heating and cooling, the type of oil used, and the thickness of the cooked food (Bignardi et al., 2019; Martinez et al., 2019). The levels of temperature, time and moisture change according to the purpose, and the most important challenge is to be able to maintain suitable cooking parameters according to the consumer needs, to provide their ideal flavour and colour. In sliced potatoes (i.e., crisps), the cooking temperature should not exceed 168 °C, and the final moisture content must not be <1%, while for other potato products the temperature depends on the type of recipe, and is in any case around 175 °C (FooddrinkEurope, 2019). The final colour of the food must be slightly golden, without tending to brown (Fig. 8).

(Figure 8 should be inserted here)

Pre-fried potatoes do not contain high AA levels, although these do not correspond to the final levels that will be defined following the second cooking phase (FooddrinkEurope, 2019). Such products intended for frying at home therefore need to include indications on the label about how the potatoes should be cooked. Here, the degree of final colouring has been seen to be an excellent parameter to monitor even in the home, as it has been shown that the AA levels and the colour are directly proportional; i.e., the darker the product after cooking, the higher the AA content (EU Regulation 2158/2017).

According to the new regulations issued by the EU, FBOs are required to apply effective controls during the process phases so that the food obtained will be suitable for the market both from the product and the food safety point of view. These include the agronomic choices (including the variety used), the production processes, and the information provided for the consumer. These are illustrated in Fig. 9, where it can be seen that during the production processes, different techniques can be used to lower the levels of the AA precursors.

However, further to these agronomic choices and optimisation of the production system, more and more studies are are now evaluating the use of natural substances as additives, such as polyphenols or natural extracts based on polyphenols. If these are not harmful to humans, the reasoning here is to reduce the AA levels in these fried potato-based foods. Some of these studies have shown excellent results for AA mitigation in the final products, and therefore future industrial applications of such natural additives cannot be excluded (Pantalone et al., 2021).

(Figure 9 should be inserted here)

The formation of AA in food also affects the family context, and due to the different culinary practices adopted worldwide, and even within countries and regions, it is difficult to make any concrete risk assessments. Indeed, a recent survey by Mesias et al. (2020a) of 730 Spanish families emphasised the many variables that can come into play during frying at home. Most families, but not all, know and choose the variety of the available products they use for frying. However, the washing, peeling and cutting phases of the potato preparation are often carried out in different orders; moreover, the soaking step in water, which is important to reduce the content of AA precursors, is not always carried out. In most cases, this phase is included mainly for hygienic processes or to avoid oxidation of the potatoes while other dishes are being prepared. During the frying phase, it was not possible to investigate with certainty the temperatures of the oil used, but in most families, the preference for the final colour of the potatoes was indeed ‘golden’ (Mesias et al., 2020a). The exposure of the final consumer to AA can vary by up to 80% depending on the frying conditions, which is why the general population must be made aware of the actual dangers, and learn to apply the right preparation methods.

Also, in a further study by Mesias et al. (2021) that involved controlled and randomised tests on a population sample, they noted that the variable that most affected the production of AA to levels greater than the reference levels (i.e., >500 μg/kg) was actually the frying time, if this exceeded 85 s.

In the study by Mesias et al. (2015), they evaluated the AA content in 70 potato-based snacks that were marketed in Spain by 33 different producers. This analysis took place 1 year after the introduction of the EU Regulation, and was carried out to assess the current state of the potato snack industry and the grade of compliance with the EU regulations.

The AA contents in the potato chips on the market analysed in this study were compared with data previously obtained by the same research group in 2015 (Mesias et al., 2015), in which they had evaluated the trend from 2004 to 2014. In general, the data showed that the mean AA content in 2019 was 55.3% lower than in 2004, and 10.3% lower than in 2008, although also very similar compared to 2014. However, 27% of the samples analysed showed concentrations higher than the reference level established in the the EU regulations (750 μg/kg). Therefore, although the mitigation measures implemented by Spanish producers have been shown to be effective, companies must continue their efforts to reduce the formation of AA in this food product, to obtain the lowest possible levels, also considering the levels established by EU Regulation 2158/2017 (Mesias et al., 2020b).

## Bread, breakfast cereals and baked goods

Cereals were the first plants used by humans in the food sector, and they are the main energy source due to their high content of carbohydrates. Exposure to AA through these products is linked to the different dietary habits of the various countries (Zilic et al., 2020).

In wheat, rye and other cereals, AS is the predisposing factor for the formation of AA, and therefore limiting its levels is the first step for the reduction of AA in the final products (Curtis et al., 2018). In many plant species, AS represents an important nitrogen storage and transport molecule, which together with glutamine and glutamate, makes up 70% of the amino acids of wheat. AS reaches high concentrations during seed germination and in response to various biotic and abiotic factors, such as sulphur deficiency and the development of diseases. Nitrogen fertilisation promotes the accumulation of AS (Raffan and Halford, 2021).

Sulphur increases the yield of crops and guarantees that the protein content of the grain is suitable for bread making (i.e., ~13%). In a study on whole flours from winter wheat varieties, a sulphur deficiency resulted in increased levels of free AS (from 5 mmol/kg to 153 mmol/kg) and other amino acids. The lack of sulphur does not allow the correct use of nitrogen to produce the reserve proteins, and consequently the plant accumulates AS. Therefore, to keep the AA content under control, a balanced fertilisation with sulphur is of fundamental importance, without exceeding the quantities of nitrogen (Raffan et al., 2020; Fooddrinkeurope, 2019). In addition, it is important to ensure the correct use of plant protection products, in terms of the control of fungal diseases, which can promote increased levels of free AS (EU Regulation N° 2017/2158).

As shown in Fig. 8 relating to potatoes, the production of safe baked goods also requires suitable agronomic and process choices for the control of the formation of AA. The final AA content can be affected here by the choice of plant variety, the genotype of the plant, the environmental factors, and the identification of genes that lead to the production of AS (Curtis et al., 2014; Raffan and Halford, 2021).

In cereals, AS is mainly contained in the outermost layers of the kernel, and in the bran and aleurone layers, which are preserved more in wholemeal flours (Zilic et al., 2020). Any recipe must take into account the type of flour used, noting also that there may be variations in the nutritional value and sensory characteristics (FooddrinkEurope, 2019).

The use of low levels of the asparaginase enzyme can effectively mitigate the levels of AA in the finished product. The effects here will depend on the conditions in terms of moisture, temperature, time and distribution in the dough (FoodrinkEurope, 2019).

The cooking phase of the various cereal products is a very important stage for achieving the characteristics of flavour, colour and aroma required by consumers; therefore, there remains the key objective for the lowering of AA levels without reducing the organoleptic properties. Capuano et al. (2008) studied the effects of cooking on the production of AA using thin strips of bread. It was seen that during cooking, loss of water occurs with an exponential increase in temperature; in the initial phases, the loss of water is faster, and the browning will become more intense. Darkening is linearly correlated with the loss of water and with the formation of AA.

Instead, Suman et al. (2020) studied a model of AA formation in wholemeal flour biscuits and cocoa biscuits, to determine how the different parameters act in the production of AA. They noted that pH was the most important variable to keep under control, as its increase was responsible for acceleration of the reaction between reducing sugars and AS. For the ingredients, the dextrose (glucose) content used to improve the consistency of the products contributed to the increase in AA levels by up to 120% when using high cooking temperatures (200 °C for 8 min). The effects of dextrose for increased formation of AA was mitigated by lowering the temperature to 180 °C, where the levels of AA were decreased by up to 77%. They also noted that the choice of suitable sugars, and the addition of amino acids and protein-based ingredients can reduce the formation of AA (Suman et al., 2020).

The addition of reducing sugars in biscuits or gingerbread is important for the final flavour, so not using these in order to reduce AA levels will mean losing sensory characteristics that are important for the sale of the product (FooddrinkEurope, 2019). Instead, combining different flours can be a simple and useful way to reduce AA levels. Rye is the cereal that develops the highest amounts of AA, followed by wheat, corn and finally rice (although there is also the need to pay attention to arsenic levels, especially in baby products) (FooddrinkEurope, 2019).

Table 4 shows the mitigation strategies reported by FooddrinkEurope in their latest toolbox, along with results from other studies, in terms of the process strategies designed to reduce the levels of AA in the final products. Of note, it is very difficult for companies to manage the recipes and the levels of AA, and there are no univocal protocols; rather, *ad-hoc* plans must be studied for the protection of the population. The design of production processes must also take into account the possible added heat treatments to the co-ingredients, such as nuts, dried fruit, cocoa beans, and others (EU Regulation 2158/2017; Suman et al., 2020).

(Table 4 should be inserted here)

In Croatia, a study by Andacic et al. (2020) compared the AA levels in different types of bread and other baked goods. For a total of 100 samples analysed, they provided a comparison of before and after EU Regulation 2017/2158. Their results showed that between 2015 and 2016, of the 35 bread samples analysed, four (11.4%) exceeded the indicative values for AA in EU Regulation 2013/647 that was valid at that time: spelt bread (81 μg/kg), rye bread (237 μg/kg; 161 μg/kg) and potato bread (227 μg/kg). Of the 32 bread samples analysed between 2017 and 2018, only one exceeded the indicative AA levels: mixed rye bread with sunflower seeds (165 μg/kg). After EU Regulation 2017/2158 came into force, 23 samples were analysed. Here, although the reference values set for bread in 2017 were 30% lower than the previous indicative values, none of the samples actually exceeded the reference values; indeed, all of the samples showed lower AA contents than in the previous years. This indicates that the manufacturers (i.e., FBOs) had started to apply the measures that were aimed at reducing AA in this food category (Andacic et al. 2020). To better illustrate this concept, in Fig. 10 we show 6 images, each one with a different colour intensity due to the different degree of roasting, which can then be correlated with the amount of acrylamide present.

(Figure 10 should be inserted here)

In a study carried out in Romania, 46 cereal-based foods were analysed between 2018 and 2019, as bread, crackers and biscuits. The aim was again to assess compliance with the limits imposed for this food category in EU Regulation 2158/2017. Bread is one of the most consumed cereal-based products in the Romanian diet, with Romania as one of the top three bread-consuming countries in Europe (FRD, 2016). Here, 19 samples of commercial bread were analysed, and none of these exceeded the indicated AA limits of 50 μg/kg for wheat-based bread, and 100 μg/kg for the other types of bread.

Between 2018 and 2019, for three samples of Lebanese bread of the same brand that were analysed, as one in 2018 and two in 2019, Mihai et al. (2020) reported a 30% reduction for the AA levels in 2019, which suggested that mitigation measures had been applied by the FBO to reduce the AA in this product. Six samples of the cracker category were analysed, and none of these exceeded the limits imposed by the regulations. For the biscuit and wafer category, 13 samples were analysed between 2018 and 2019, and here four samples of biscuits exceeded the reference AA levels (350 μg/kg). However, the analysis of one of the biscuit samples that was repeated a year later showed a 70% decrease in the AA level (Mihai et al. 2020).

An earlier study by Mesias et al. (2019) also considered the category of biscuits, where they evaluated the AA content in 80 different samples marketed in Spain. These were compared with data obtained for the same food category in 2007 by the same research group. The results obtained showed that 30% of the samples analysed exceeded the reference levels required by EU Regulation 2158/2017. Then, on the basis of these comparisons between 2007 and 2019 for the AA levels in the Spanish biscuit market, these declined by 45% for classic wheat-based biscuits. For a general comparison across all of the types of biscuits analysed in 2019, which also included some biscuits with new formulations of different types of cereals and pseudo-cereals that were not available in 2007, and with the exception of two samples based on oats and rice, there was only a small decrease in the AA content (18%), which did not reach significance. These details require further attention from the relevant administrative bodies for food safety and the biscuit industry (Mesias et al., 2019).

In Slovenia, a study was carried out to determine the AA contents across different food categories; namely, salty snacks, biscuits and wafers, bread and coffee, with the aim of monitoring the exposure of the Slovenian adult population to AA and the fulfilment of EU Regulation 2158/2017. In total, 41 food samples were analysed; where the relevant AA levels were exceeded by 86% for bread, 80% for breakfast cereals, 57% for biscuits, and 31% for savoury snacks (Mencin et al., 2020). These findings highlight the need for more monitoring and health education that should be aimed at both the producers (i.e., FBOs) and the consumers, to reduce the AA consumption in Slovenia in particular, as well as on an international basis.

## Coffee and its substitutes

Together with tea, coffee is the most consumed beverage in the world after water (Esposito et al., 2020). Coffee is consumed annually by about 40% of the population, with consumption of around 400 billion cups per year (Zawirska-Wojtasiak et al., 2018). Given the high consumption, the presence of AA as an unwanted compound therefore represents a very important problem.

To obtain coffee, the seeds that are extracted from the drupe are used (commonly called coffee grains, or beans), which are subjected to a roasting process, with the aim being to create a unique aroma, colour and taste. Generally, the roasting phase takes place at 200 °C for <20 min, but in reality, the production process will include specific ranges of temperatures and times to obtain the desired characteristics of the coffee. Indeed, different countries have different habits and choices for their coffee. In southern Europe, a medium-dark roasted coffee is preferred, while in America and in northern Europe, the preference is for lightly roasted coffee (Schouten et al., 2020). These differences are based on the following:

* With light roasting at temperatures of 170-190 °C, a coffee with a sweeter and more acidic taste is obtained;
* With medium roasting at temperatures of 200-220 °C, a coffee with a balanced flavour and aroma is obtained;
* With dark roasting at temperatures of 230-240 °C, the coffee will have a strong and bitter taste.

The roasting process of green coffee beans comprises several phases. These include initial dehydration, with an increase in volume and a decrease in weight of the beans. This is followed by the steps that involves the Maillard reaction, here as the caramelisation reaction and pyrolysis. Consequently, the organic molecules undergo transformation, with the formation of many compounds that contribute to the aroma, colour and flavour of the coffee (Esposito et al., 2020; Toci and Farah, 2014). Indeed, following the roasting, the polysaccharide chains in coffee are degraded, which releases simple sugars. Together with the sugars already present and the hydrolysed sucrose, these simple sugars go through the Maillard reaction and Strecker degradation (from which aromatic substances are obtained; e.g., ketones, aldehydes, pyrazines), and the caramelisation process. The sugars also undergo fragmentation, with the formation of CO2, aliphatic and cyclic hydrocarbons, and short-chain organic acids, in particular. The terminal phases of the reactions here promote the formation of melanoidins, which determine the final colour of the coffee.

For coffee, the colour is an important indicator of the degree of roasting (Fig. 11), and it is directly related to the organoleptic properties of the final product (FooddrinkEurope, 2019).

(Figure 11 should be inserted here)

Bertuzzi et al. (2020) studied the formation of AA during the coffee roasting and reported that it is formed during the first few minutes of the roasting process. The maximum AA levels were reached after 10 min at 175 °C to 177 °C, as 1045 ±56 μg/kg for Arabica beans, and 795 ±45 μg/kg for Robusta beans. At the end of the process, the AA levels were actually much lower than in the initial phase, at ~300 μg/kg, which suggested that at temperatures above 210 °C the AA levels in coffee beans tend to decrease (Bertuzzi et al., 2020). However, the mechanism by which these AA levels decrease is not yet known, although evaporation or reactions with melanoidins have been proposed (Badoud et al., 2020; Pastoriza et al., 2012). In the final product (i.e., roasted coffee beans), the final AA levels represent 20% to 30% of the initial AA levels (FooddrinkEurope, 2019).

In addition to roasting time and temperature, the AA levels in coffee depend on the concentrations of its precursors, which vary greatly according to the botanical species. AS is the limiting factor here, while the amounts of reducing sugars do not show any correlations with the AA produced (Bagdonaite et al., 2008; FooddrinkEurope, 2019).

Very recently, Schouten et al. (2021) also evaluated how roasting of Arabica and Robusta coffee beans affected the content of not just AA, but also other bioactive compounds, including chlorogenic acids and their antioxidant effects. They noted that the levels of reducing sugars were higher in Arabica, while the levels of AS were higher in Robusta (800 ±50 μg/kg *versus* 540 ±40 μg/kg). This shows that as reported by other studies, AS is indeed the predominant factor that determines the final AA levels (Shouten et al., 2021; Bagdonaite et al., 2008; Bertuzzi et al., 2020). Furthermore, this study by Shouten et al. (2021) showed that Robusta had a higher antioxidant activity than Arabica, and that the chlorogenic acids in general tended to decrease with an increase in roasting temperature.

Medium-light roasted coffee tends to have higher AA levels due to the lower temperature used during the roasting process (Badoud et al., 2020; Shouten et al., 2020). Then, under the same roasting conditions, the Robusta species has higher AA levels, due to its chemical composition (Bagdonaite et al., 2008). Of note here, the AA in coffee beans is almost completely extracted due to its solubility in water, which also means that lower AA levels have been reported for ‘espresso’ coffee due to the reduced extraction time (Gokmen, 2015).

As coffee is a concentrate of a range of compounds with antioxidant and stimulating actions, these might provide beneficial effects against diabetes and cancers. Indeed, in a recent study, Nehlig and Cunha (2020) indicated that these beneficial actions can actually offset the risks of the associated AA intake.

European Union Regulation N° 2158/2017 indicates that for the FBOs, their critical control of the roasting conditions needs to focus on temperature and time, to lower the AA levels as much as possible. Moreover, the FBOs can use the enzyme asparaginase where possible, although the potential difficulty for this treatment is that the asparaginase cannot cross the grain wall (FooddrinkEurope, 2019). However, recent tests of a combined pre-treatment with steam and asparaginase showed reduction of the AA levels by up to 59%, without affecting the further bioactive compounds in the coffee (Correa et al., 2020).

The toolbox of FooddrinkEurope (2019) highlights the difficulties for the definition of the most effective process conditions to reduce AA levels in coffee. In particular, these conditions also need to allow acceptance of the product by the consumers, as the AA is formed in the same phases as the constitution of the sensory molecules is established.

As already explained in previous section (bread, breakfast ......), during the fertilisation phases for coffee plants, the nitrogen and sulfur levels must be monitored, along with the biotic and abiotic stresses that affect the AS levels. Therefore, it is the task of the FBOs to follow good agricultural and phytosanitary practices (FooddrinkEurope, 2019; EU Regulation 2017/2158).

Of note also, there is again the study carried out by Mencin in 2020 in Slovenia, already mentioned before, where 41 food samples were analysed, including savoury snacks, biscuits and wafers, bread and coffee. In this study, only the AA levels in coffee met those required by the EU regulations, while as indicated above, for all of the other foods included, the required AA levels were far exceeded.

Finally, with coffee substitutes mostly based on cereals (barley) or vegetables (chicory), these also need to be controlled for AA levels. Again, it is essential to keep the AS content under control as a mitigation strategy for AA.

## Baby foods

The nutrition of children has a fundamental role, especially in the first 1000 days (~3 years) of their life. If their diet at this time is not well balanced, this can favour the development of diseases from infancy to adulthood. In particular also, their caloric intake is much higher per kilogramme body weight, so the correct choices and preparation procedures for their food are of great importance.

The EU Directive 2006/125/EC defines the regulation of processed cereal-based foods and other foods intended for infants and young children. These foods include two main categories, with various sub-categories, as follows:

* Cereal-based foods:
* Simple cereals, which are reconstituted or to be reconstituted with milk or another appropriate liquid;
* Cereals with the the addition of a food rich in protein, which are reconstituted or to be reconstituted with water or other liquids that do not contain protein;
* *Pastina* (finely cut pasta), that is used after cooking in boiling water or in another suitable liquid;
* Biscuits and rusks, which can be as they are or after being crumbled and combined with water, milk or another suitable liquid.
* Baby foods other than grain-based foods.

Since these foods are prevalent in the general diet of a child, it is understood that the exposure to AA can be higher than for an adult. In the latest EFSA report, the intake of AA with ‘processed grain-based foods’ was estimated at up to 30%, while that with ‘processed non-grain foods’ was up to 60% (EFSA, 2015). Children are therefore a high risk category for AA intake, while their metabolic and detoxifying systems against toxic agents will also be less efficient than those of adults. Therefore, it is essential to use contaminant mitigation strategies in this particular food category.

In this case too, the European food industry provides guidelines based on the obligations imposed by the new EU regulations. Also in this case, the control of the precursors is of fundamental importance, and of AS in particular in cereals, as well as the parameters of temperature, time and humidity in the food preparation. This must also be applied in a context of compliance with the rules on microbiological safety, and in compliance with the sensory characteristics and shelf-life of the products (EU Regulation 2017/2158).

The use of asparaginase as a mitigation strategy in cereal-based products for children is effective. The preparation of these recipes involves the use of large amounts of water, in which the action of this enzyme is facilitated, with AS reductions of up to 80% reported (FooddrinkEurope, 2019).

For further AA mitigation, it is also important to reduce the addition of reducing sugars in the various preparations (e.g., with honey, fruit, fructose), which increase the AA content in the final product (Fooddrinkeurope, 2019). As previously mentioned (section 1.2.), Becalski et al. (2011) showed that in systems rich in reducing sugars and AS, such as plums, and under conditions of drying or heating for prolonged periods, AA can form even at temperatures below 100 °C. Therefore, during the production of foods based on potatoes or fruit that are rich in precursors (e.g., prune puree), the process conditions must be modified to limit AA formation. Of note, prolonged boiling or pasteurisation can also lead to higher levels of AA; the thermal input parameters and moisture conditions must therefore be controlled (FooddrinkEurope, 2019).

# Conclusions

In this work, the problem of A in food was examined. It is a process contaminant that has toxic effects that are linked to the induction of oxidative stress, gene mutations and cytotoxic effects. These, in turn, can lead to alterations in gene expression and cell proliferation and differentiation, and are associated with the development of neurological damage, cancers and adverse effects on the foetus.

Following the indications provided by EU Regulation 2158/2017 that is designed to minimise the production of AA in foods, the obligations of the FBOs can be grouped into several salient points:

* the agronomic choices, for the selection of raw materials and new varieties with reduced levels of AA precursors;
* the fertilisation phases during crop production;
* the storage times and storage conditions of the raw materials;
* the temperature, time and humidity parameters;
* the additives used, which can reduce or block the reaction between AA precursors;
* the final colour of the food.

Looking at the levels of AA found recently in some industrially produced foods, it emerges that these have undergone sharp decreases since the discovery of the problems of AA and this was accepted by the European Commission which provided to revise the previously published "indicative values" of some food typologies, emitting new "benchmark levels" as reported in Table 5 (EFSA CONTAM 2015; EU Regulation N° 2158/2017).

(Table 5 should be inserted here)

They appear to have reached their lowest levels about 10 years after this discovery, and from that moment on the trends have remained almost constant. This means that in general, food companies have indeed carried out changes to their production processes to lower AA levels, although there remains the need for further effort. In this sense we want just to show the example of potato crisps that represent a well-documented food typology, to make into evidence the successes of the efforts made during the years in the mitigation of AA (Raffan and Halford, 2019). Plotting the mean values of AA versus the years it is evident the presence of two different slopes in the range respectively 2002-2011 and 2011-2019 (Fig. 12). Furthermore it is also worthy of note the fact that the mean values reported by the European Snacks Association (ESA) and the same reported by EFSA are not properly superimposable since the EFSA values are noticeably higher. Nevertheless we can equally say that, in both cases, the trend goes down and this is, in any case, positive for the safety of the product.

Future research areas should focus on the combination of mitigating technologies and techniques that can be used to reduce the formation of AA, to thus be able to provide food products that are safer for human health.

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# Data availability statement

Data sharing is not applicable to this article as no new data were created or analysed in this study

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Captions for manuscript: "Acrylamide in widely consumed foods"

Figures

Figure 1

Chemical structure of AA

Figure 2

Reaction pathway of AA production (adapted from Khan et al., 2019)

Figure 3

AA content in various food categories (data from EFSA, 2015)

Figure 4

Relative contributions of foods in the diet in terms of the exposure of the adult population to AA (data from EFSA, 2015)

Figure 5

Phase I metabolism of AA in the liver (pathway (a)) and the DNA adduct N7-glycidamide-guanine (pathway (b))

Figure 6

Schematic representation about the toxicity of AA

Figure 7

Metabolic pathway of AA in humans; on the right side we depicted the main four metabolites detected in human urines (Wang et al, 2017; Zhang et al., 2020)

Figure 8

Acceptable (left) and non-acceptable (right) final colours for French fries

Figure 9

Operational procedures required from food business operators for the control of AA levels in their final products

Figure 10

A comparative image showing six browning grades of toasted bread surface: as quantitatively demonstrated in the reference by Andacic at al, 2020, the browning degree is strictly correlated with the AA content

Figure 11

A comparative image showing three samples of coffee beans according to increasing roasting conditions

Figure 12

AA in samples of potato crisps over years: data were provided by European Snacks Association (ESA) (brown marks: from 2002 to 2019) and EFSA (blue marks: from 2011 to 2018); it was marked the difference between the two slopes of the ESA data acquired before and after 2011.

Caption for Tables

Table 1

Chemical-physical characteristics of AA

Table 2

Regulation of AA levels in food, as defined by European Commission EU Regulation N° 2158/2017

Table 3

Further potential food sources of AA, as defined by the recent EU recommendations (N° 2019/1888)

Table 4

Process strategies for mitigation of AA in baked goods (FooddrinkEurope, 2019 and other studies)

Table 5

"Indicative values" and "benchmark levels" for AA in several food typologies set by the European Commission

Figure 1

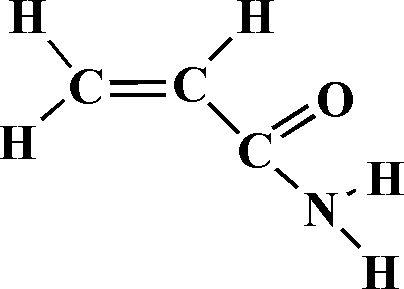


Figure 2

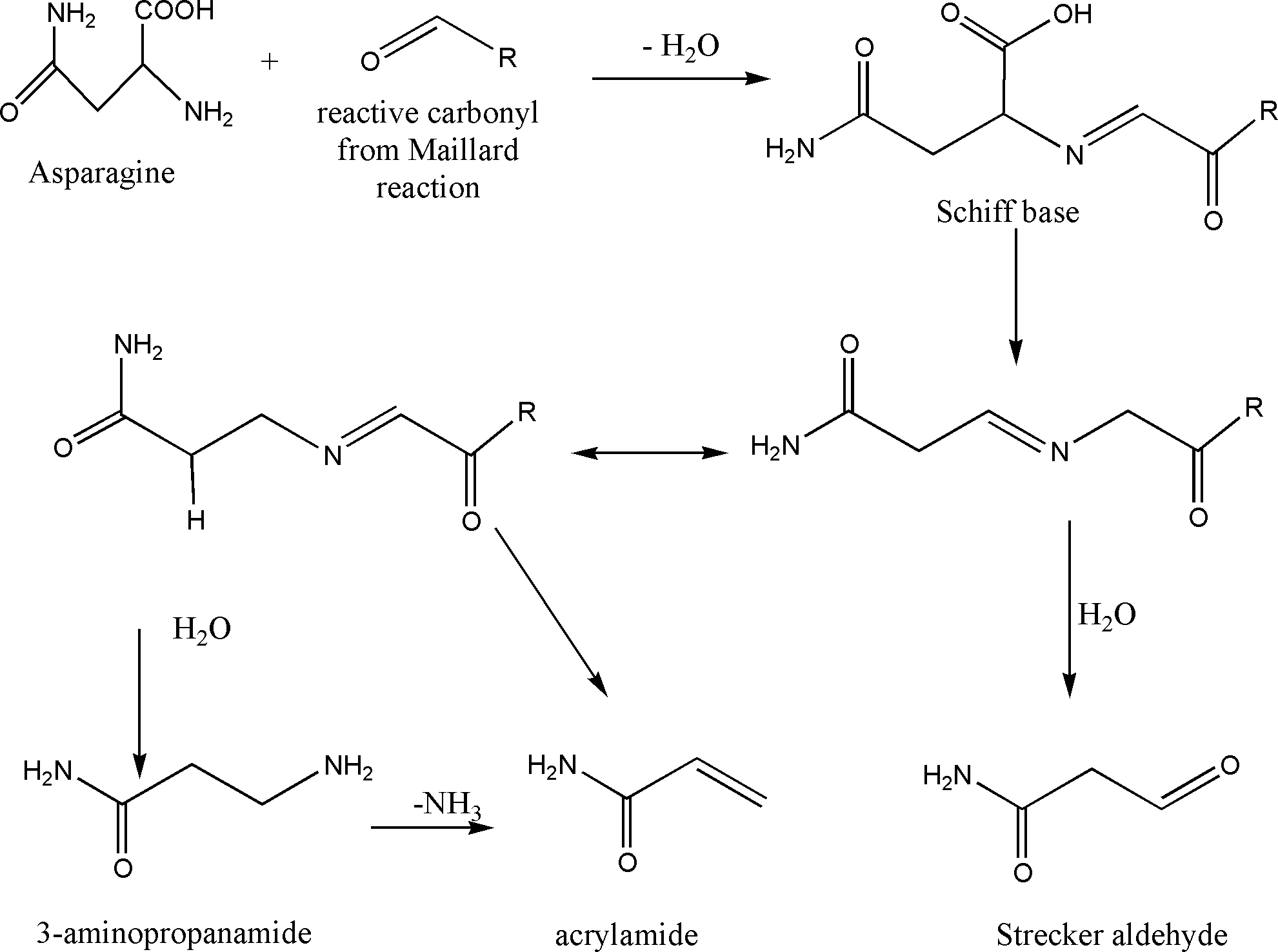


Figure 3

Figure 4

Figure 5

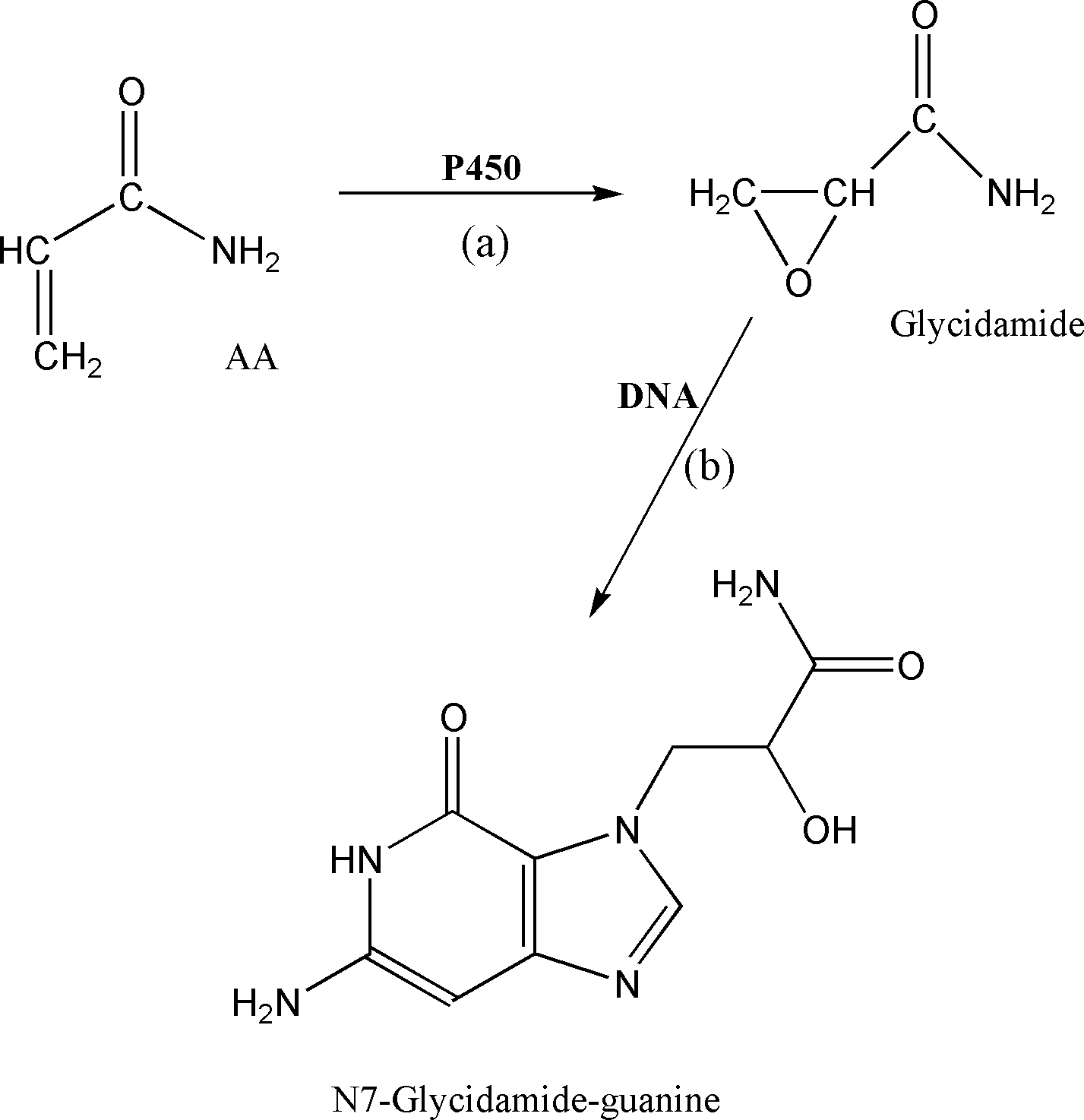


Figure 6

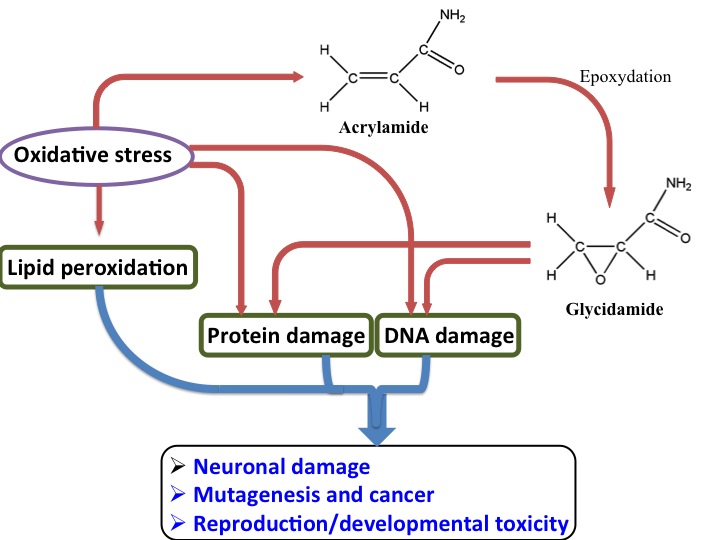


Figure 7



Figure 8



Figure 9

|  |  |  |
| --- | --- | --- |
|  |  | * Choice and selection of varieties with lower levels of reducing sugars and asparagine * Control of the acrylamide precursors and correct use of fertilisers * Harvesting of potato tubers when they are ripe |
|  |  | * Choice of potato varieties that are suitable for the process, and control of the precursor levels (i.e., reducing sugars and asparagine) * Control of storage conditions for temperature and misture, to avoid sweetening due to aging and for suppression of shoot formation * Monitoring of precursor levels |
|  |  | * The operations vary according to the intended form of the final product * Analysis of reducing sugars before the production process (e.g., frying test) and elimination of tubers that are not ripe * Immersion of potato sticks in hot water (blanching) at temperatures and times defined according to the processing, to lower the levels of precursors * -Use of asparaginase, antioxidants, amino acids and calcium salts to control the precursor levels and the colour of the final product * Control of the frying parameters of oil temperature and final moisture of the product * Evaluation of the final color (Fig. 6) |
|  |  | On the packaging and/or through various communication channels, the recommendations should be to:   * Preheat and keep the frying temperature between 160 °C and 175 °C, or the baking temperature between 180 °C and 220 °C * Achieve a final ‘golden yellow’ colour of the potato * Turn baked goods after 10 minutes, and do not overfill the deep fryer * Do not overcook the potatoes |

Figure 10

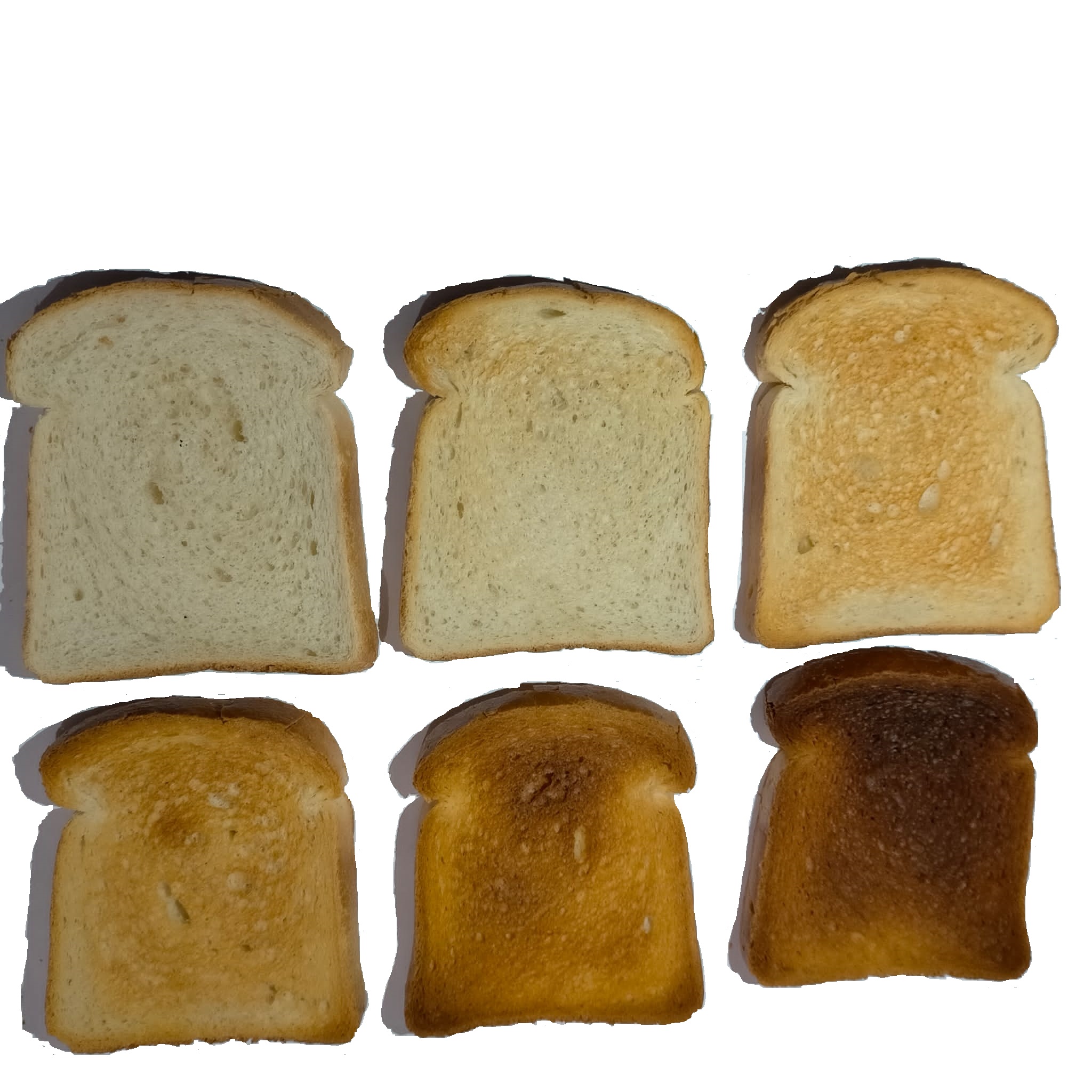


Figure 11



Figure 12

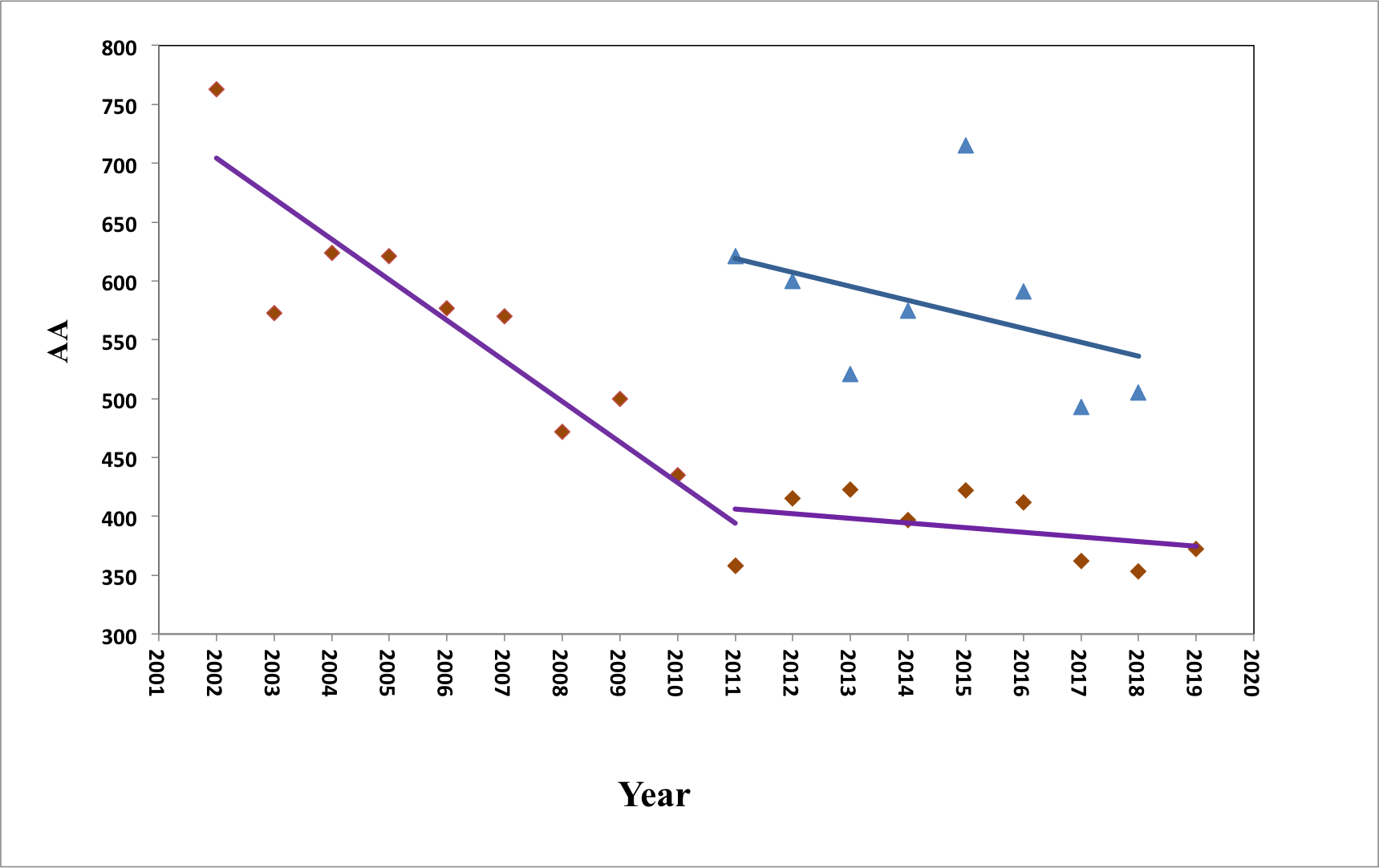


Table 1

|  |  |
| --- | --- |
| **Characteristic** | **Detail** |
| CAS Number | 79-06-1 |
| Molecular weight | 71.08g/mol |
| Melting point | 84.5°C |
| Boiling point | 192.6°C |
| Density (at 30 °C) | 1.12g/mL |
| Stability | Stable at room temperature |
| pH (50% aqueous solution) | 5.0-6.5 |
| High solubility solvents (at 30 °C) | Water: 2.155 g/mL |
|  | Methanol: 1.55 g/mL |
|  | Ethanol: 0.862 g/mL  Acetone: 0.631 g/mL |
| Low solubility solvents (at 30 °C) | Chloroform: 0.027 g/mL |
|  | Benzene: 0.003 g/mL |
| Partition coefficients | Log Kow: -0.67 |
|  | Log Koc: 1 |
| Henry’s Law constant (at 25 °C) | 1.7 ×10-9 atm-m3/mol |

Table 2

|  |  |
| --- | --- |
| **Food** | **Benchmark (µg/kg)** |
| French fries (ready-to eat) | 500 |
| Potato crisps from fresh potatoes and from potato dough | 750 |
| Potato-based crackers |  |
| Other potato products from potato dough |  |
| Soft bread |  |
| Wheat based bread | 50 |
| Soft bread other than wheat-based bread | 100 |
| Breakfast cereals (excluding porridge) |  |
| Bran products and whole grain cereals, gun puffed grain | 300 |
| Wheat and rye-based products | 300 |
| Maize, oat, spelt, barley and rice-based products | 150 |
| Biscuits and wafers | 350 |
| Crackers with the exception of potato-based crackers | 400 |
| Crispbread | 350 |
| Spiced bread (ginger bread) | 800 |
| Products similar to the other products in this category | 300 |
| Roast coffee | 400 |
| Instant (soluble) coffee | 850 |
| Coffee substitutes |  |
| Exclusively from cereals | 500 |
| Exclusively from chicory | 4000 |
| Baby foods, processed cereal based foods for infants and young children, excluding biscuits and rusks | 40 |
| Biscuits and rusks for infants and young children (as defined in Regulation (EU) N° 609/2013) | 150 |

Table 3

|  |  |
| --- | --- |
| **Food group** | **Food** |
| Potato products | Roast potato |
|  | Croquettes, pommes duchesse, pommes noisettes |
|  | Potato (and vegetable) casserole |
|  | Potato and meat meal |
|  | Potato and cheese meal |
| Baked products | Rolls (hamburger, whole wheat, milk) |
|  | Pita bread, Mexican tortillas |
|  | Croissants |
|  | Doughnuts |
|  | Speciality bread (pumpernickel bread, ciabatta with olives, onion bread) |
|  | Pancakes |
|  | Crisp cookies (deep fried thin strips of dough) |
|  | Churros |
| Cereal products | Rice crackers |
|  | Maize crackers |
|  | Cereal snacks (such as extruded maize and/or wheat products) |
|  | Honey roasted muesli |
| Others | Vegetable crisps/fries |
|  | Roasted nuts |
|  | Roasted oilseeds |
|  | Dried fruit |
|  | Roasted cocoa beans and derived cocoa products |
|  | Olives in brine |
|  | Coffee substitutes not based on chicory or cereals |
|  | Fudge, caramel, nougat |

Table 4

|  |  |  |
| --- | --- | --- |
| **Mitigation strategy** | **Effects on final product** | **Source reference** |
| Flour combinations | Lower AA levels due to lower starting AS content (but potentially effects on flavour and texture) | Žilić et al., 2020 |
| Replacement of fructose with glucose | Reduction in the AA levels, especially in recipes with ammonium bicarbonate | FooddrinkEurope, 2019 |
| Replacement of ammonium bicarbonate with sodium bicarbonate and acidifiers | Food business operators must ensure that the organoleptic properties are not changed | EU Regulation 2158/2017 |
| Addition of lactic acid bacteria | By producing organic acids in bread, the pH is lowered, thus reducing AA levels | Albedwawi et al., 2021 |
| Addition of cysteine and glycine to the dough | Competition with AS for the reaction with the sugar fraction, so less final AA formed | Zou et al., 2015 |
| Addition of pectin or citric acid | Lower pH | Passos et al., 2018 |
| Addition of antioxidants | Might inhibit formation of AA | Yang et al., 2019 |
| Lower cooking temperature for longer time | The lower temperature promotes less AA formation | FooddrinkEurope, 2019 |
| In leavened breads, fermentation times are increased | Reduction of AA levels, attention to the final texture of the bread | Mustafa et al., 2009 |
| Control of the final colour | The final colour of the product must be golden yellow, and not tending to brown | FooddrinkEurope, 2019 (Fig. 9) |
| Consumer information | Food business operators must provide information for baking bread if it is to be completed in the home | EU Regulation 20158/2017 |

Table 5

|  |  |  |  |
| --- | --- | --- | --- |
| **Food** | Indicative value **2011** (ppb) | Indicative value **2013** (ppb) | Benchmark level **2017** (ppb) |
| French fries | 600 | 600 | 500 |
| Potato crisps | 1,000 | 1,000 | 750 |
| Soft bread (wheat) | 150 | 80 | 50 |
| Soft bread (other) |  | 150 | 100 |
| Breakfast cereals: bran products, whole grain cereals, gun puffed grain | 400 | 400 | 300 |
| Breakfast cereals: wheat and rye based |  | 300 | 300 |
| Breakfast cereals: maize, oat, spelt, barley and rice based |  | 200 | 150 |
| Biscuits | 500 | 500 | 350 |
| Crackers | 500 | 500 | 400 |
| Crispbread | 500 | 450 | 350 |
| Gingerbread | -- | 1,000 | 800 |
| Cereal-based baby foods | 100 | 50 | 40 |
| Baby foods (not cereal based) without prunes | 80 | 50 |  |
| Baby foods (not cereal based) with prunes |  | 80 |  |
| Biscuits and rusks for infants and young children | 250 | 200 | 150 |
| Roast coffee | 450 | 450 | 400 |
| Instant coffee | 900 | 900 | 850 |
| Coffee substitute (cerealbased) | -- | 2,000 | 500 |
| Coffee substitute (chicory) | -- | 4,000 | 4,000 |