Chapter 42
Risk Assessment of Human Carcinogenicity of Acrylamide in Food: Way to Reduce the Predicted Mitogenic Side Effects Through Mitigation Strategy

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Abstract Acrylamide (AA) is probably carcinogenic to humans that may have an impact on human cancer risks. The World Health Organization (WHO) consultation endorsed the International Agency for Research on Cancer (IARC) classification of AA for Group 2A (probable human carcinogen). Everyone is exposed to AA through diet and it is thought that it may be genotoxic and cause cancer via its conjugation with glutathione (GSH) that can lead to depletion of cellular GSH stores, altered redox status of the cell and affect genes expression through regulation of various redox-dependent transcription factors. It has been shown that AA and its metabolite, glycidamide, may be critical for carcinogenic and genotoxic properties, where they form *in vivo* and *in vitro* DNA damage. Analysis of blood and urinary biomarkers of AA exposure needs to be calibrated and their correlation to dietary intakes should be investigated. Although only a limited number of starchy foods have been analyzed for AA content, no data are available for foods consumed in many countries particularly in developing countries. Addition of essential amino acid, lysine, along with antioxidant, ascorbic acid can inhibit or mitigate AA risk in foods.

Keywords Acrylamide · Cancer · Genotoxic · Glycidamide · Mitigate

42.1 Introduction

Acrylamide (AA) or 2-propenamide (AA, C_3H_5NO) is a white crystalline solid that soluble in water, with a relative molecular weight of 71.08 kDa, and commonly used in the industry. For decades, AA has been used for polyacrylamide polymer production, which has many industrial applications as a coagulant in the treatment of water, cosmetics, conditioning of soil, additives in papermaking; grouting material for

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O. O. Babalola (ed.), *Food Security and Safety*,
https://doi.org/10.1007/978-3-030-50672-8_42

dams, tunnels, and other underground building constructions and in biomolecular laboratories for use in electrophoresis gels. Exposure to AA was previously thought to occur through industrial activities or cigarette smoking, as the compound is also found in tobacco smoke (Rong et al. 2013; Wuethrich et al. 2014; Wei et al. 2015; Lenze et al. 2016). But, recently AA was found to be detected in many different types of consumed foods around the world. Increased exposure to AA was studied to elevate the probability of carcinogenicity in humans and has an impact on cancer risks. In the present chapter, we discuss the formation of acrylamide (AA) in food products, its consumption, and its possible carcinogenic risk. The present and future mitigation strategies that may reduce the exposure to AA are also discussed. The present chapter may be beneficial for scientific researchers, the food industry, and medical personnel.

42.2 Acrylamide in Food Products

In the year of 2002, AA was detected in heat-treated foods where its formation was dependent on temperature. Moderate levels of AA (5–50 μg/kg) were detected in heated foods rich with protein using liquid chromatography-mass spectrometry (LC-MS), but higher contents of AA (150–4000 μg/kg) were detected in foods rich with carbohydrates. Importantly, AA couldn't be detected in unheated or boiled foods (Tareke et al. 2002). It was found that, AA in food could be formed by high-temperature cooking *via* different mechanisms, i.e., formation *via* acrylic acid which may be derived from the lipid degradation, carbohydrates, or free amino acids; formation *via* the dehydration/decarboxylation of organic acids such as malic acid, lactic acid, and citric acid; and direct formation from amino acids (WHO 2002). Many studies have shown that AA is formed mainly from free amino acid, asparagines, and reducing sugars such as glucose and fructose, through high-temperature cooking by Maillard reactions. Maillard reaction is a cascade of non-enzymatic reactions between free amino acids and reducing sugars which is responsible for the flavor and color generated during baking, e.g., in cereals and potatoes (Brathen and Knutsen 2005). The mean AA contents in potato crisps prepared from different potatoes that were grown in UK were found to be ranged from 131 to 5360 μg/kg. Rosti, a popular Swiss dish of grated and fried potatoes, contains AA average of 702 μg/kg. The concentration of AA in different commonly consumed bread ranges from <limit of quantification (LOQ) to 695 μg/kg, where the highest AA concentration was observed in wheat bran and whole wheat bread. Identically, AA was also detected in different bakery products such as biscuits (LOQ to 2405.0 μg/kg), sandwich biscuits with cream (112.6–570.4 μg/kg), gingerbread (349.5–955.5 μg/kg), and crackers (347.8–366.1 μg/kg). Also, coffee beverages acquired from coffee vending machines were found to be contained AA concentration which varies from 7.7 to 40.00 μg/L. Estimated AA intakes were measured using a food frequency questionnaire, total diet study, hemoglobin adducts of AA, and glycidamide (GA, the primary metabolite of AA). The estimated mean AA intakes for adults ranged from 0.3 to 0.6 μg/kg of body weight per day, where the

intake is higher in children. Infant cereal-based foods are an important nutrient source for children around the world, and the concentration of AA in that baby foods (ready-to-eat and instant baby foods, candy bars, and cakes) varied between 10 and 60 μg/kg.

In another study, the mean AA level in cereal-based baby foods ranged from 36 to 604 μg/kg, and it was estimated the mean AA exposures of toddlers from the cereal-based baby food was 1.43 μg/kg of body weight per day (Kumar et al. 2018). The detection of AA in foods was first reported by the Swedish National Food Administration (SNFA) in 2002. Until now, there are no permissible limits set worldwide for AA consumption in the diet. Dietary AA intake may increase breast and kidney cancer risks. The daily intake of AA in human diets was estimated to be 0.3–0.8 μg/kg of body weight (Krishnakumar and Visvanathan 2014). The consumption of fast food in the current generation has led to various food behavioral changes, as the result of an improper diet daily. Although the concentration is low in dietary AA, it contributes negative effects with frequent consumption. French fries, potato chips, tortilla chips, and crispbread are the most widely consumed foods that contain AA. The source of dietary AA in fried potato accounts for about 272–570 μg/kg. The AA ranged from 75–1044, 149 and 229–890 μg/kg, for bakery products, breakfast cereals, and coffee, respectively. The mean AA concentration was found to be 399–1202, for potato chips, 159–963 μg/kg for French fries, 169–518 μg/kg for cookies and 3–68 μg/kg for coffee. Cereals of breakfast are major contributors to AA formation in Western countries due to the daily intake of it in the diet. The AA content in cereals was detected to be ranged from 62 to 803 mg/kg, with an average of 292 mg/kg. Wheat-based cereals and puffed breakfast cereals contain high amounts of AA, along with high protein content. While cereals particularly gingerbreads were reported to have a high amount of AA, at about 1000 mg/kg. Also, AA content was found to be increased in olives and dried fruits when the products were exposed to high temperatures. The AA content in coffee was found to be elevated to maximum levels during the process of roasting. Levels of AA were found to be 11.4–36.2 and 200.8–229.4 μg/L for espresso coffee and coffee blends, respectively. The AA content was recorded to be 2.0 and 4.0 μg/L for tea infusions during roasting at 160 °C and 180 °C for 30 and 15 min, respectively (Baskar and Aiswarya 2018). Only a limited number of starchy food samples has been analyzed with No data are available for many countries. These samples were not in any sense representative of the products available to consumers. The range of levels of AA found in each of the several types of food (potato crisps, breakfast cereals, etc.) is broad, there is not a good understanding of the determinants of this variability and the amount of information that has been collected on AA in other types of foods is very limited. These missing data mean that the estimates of total long-term dietary exposure that have been calculated to date are likely to be underestimated by an undetermined degree and that the extent of underestimation will vary among countries, particularly for foods consumed in developing countries, and understanding the determinants of variability in AA levels in foodstuffs, are priorities for further research. AA was found in nearly all food items analyzed so far, which raises the possibility that AA may be present in other food items not yet analyzed.

However, no data were available for many commodities, such as meat (except as part of compound foods), milk, rice, cassava, and products of soy. In addition, no data were available for processed fruits (except a single negative report for dried fruit) and vegetables (only a small number of restaurant-prepared meals). The Consultation endorsed the International Agency for Research on Cancer (IARC) classification of AA for Group 2A (probably carcinogenic to humans). The available data allowed the Consultation to make only an order-of-magnitude estimate of average long-term dietary intakes of AA in developed countries, which would be 0.3–0.8 μg/kg of body weight/day. Within a population, it is anticipated that children will generally have exposures two to three times more than adult consumers when expressed on a bodyweight basis (Rosen 2002). Developing and other countries with insufficient information for determining population-level dietary exposures to AA should consider generating interim information relevant to their circumstances and analyzing blood or urinary biomarkers of AA exposure. These biomarkers need to evaluated and calibrated, and their correlation with dietary intakes should be investigated. According to the 72nd report on Food additives by the Food and Agriculture Organization and World Health Organization (FAO/WHO), the average dietary intake of AA was estimated to be 1 μg/kg of body weight/day or 4 μg/kg of body weight/day for high consumers (WHO 2011).

42.3 Acrylamide Quantification in Food

Many commonly applied techniques are used to measuring the levels of AA in many types of foods, such as; gas chromatography-mass spectrometry (GC-MS) (Geng et al. 2011) and LC-MS with isotope-labeled internal standards (Hamide and Vural 2005). Besides, many other analytical methods are used to detect the AA contents in the foodstuffs include; capillary electrophoresis mass spectrometry (CE-MS), high-performance liquid chromatography-mass spectrometry (HPLC-MS) (DeArmond and DiGoregorio 2013), and non-aqueous capillary electrophoresis. Also, ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) with a time of flight (TOF) was also applied for the determination of AA content and its intermediates (Hu et al. 2015).

42.4 Carcinogenic Metabolism of Acrylamide in the Human Body

The absorption of AA is rapid and complete by the oral route in all species and widely distributed in all body tissues. In humans, AA is primarily metabolized in the liver to its epoxide metabolite, glycidamide (GA), by AA-metabolizing enzyme cytochrome P450, family 2, subfamily e, polypeptide 1 (CYP2E1) (Ghanayem et al.

2005a). GA may subsequently conjugate with reduced glutathione (GSH) to produce GSH conjugates by GSH transferases (GST); the conjugates are then excreted in urine as mercapturic acid derivatives, which consider biomarkers of short-term exposure to AA. In addition, GA can be further metabolized to glyceramide by epoxide hydrolase enzyme in the liver. AA and GA may also react with the N-terminal valine residue of hemoglobin (Hb) resulting in the formation of covalent adducts (hemoglobin adducts) that reflect the time-weighted exposure to AA (Ghanayem et al. 2005b; Tolgahan and Vural 2016) (Fig. 42.1). Glutathione (GSH) is a significant antioxidant that inhibits cellular damage triggered by reactive oxygen species (ROS). While GST is a group of enzymes that mainly contributed to the detoxification of xenobiotics, cell death, and cell proliferation (Kumar et al. 2018). Glutathione (GSH) is employed by GST in several cellular reactions. Thus, the enzymatic activities of GST depend upon the availability of GSH. Srivastava et al. (1986) reported that a single administration of AA decreases the GSH content with-

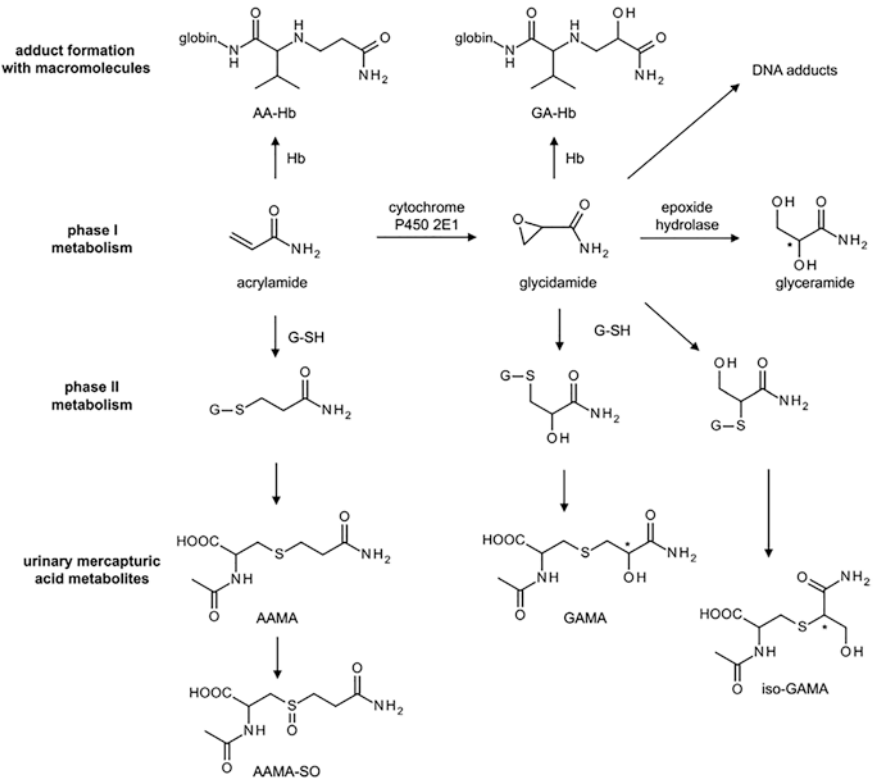

Fig. 42.1 Biotransformation of acrylamide in human. Hemoglobin (Hb); cytochrome P450, family 2, subfamily e, polypeptide 1 (cytochrome P450 2E1); N-acetyl-S-(2-carbamoylethyl)-l-cysteine (AAMA); N-acetyl-S-(2-carbamoylethyl)-l-cysteine-sulfoxide (AAMA-SO); N-acetyl-S-(2-carbamoyl- 2-hydroxyethyl)-l-cysteine (GAMA), N-acetyl-S-(1-carbamoyl-2-hydroxyethyl)- l-cysteine (iso-GAMA); * indicates chiral carbons. (Tolgahan and Vural 2016)

out affecting the activity of GST. However, repeated administration of AA (50 mg/kg × 10 days) resulted in the depletion of GSH content as well as GST activity in the brain of rats. Altered GST activity may affect the redox status of the cell and this may subsequently affect various redox-dependent genes expressions, cell transformation or proliferation, or even apoptosis (Schulze-Osthoff et al. 1995). AA's disruptive effects display different adverse effects, including cell cycle delays, heritable chromosomal translocations, chromosomal aberrations, inhibition of mitotic and meiotic processes, and aneuploidy (Kumar et al. 2018).

Nixon et al. (2012) showed that the administration of AA in drinking water of male mice at concentrations of 0.001, 0.01, 0.1, 1 and 10 μg/ml for up to 1 year, which was equivalent to 0.0001–2 mg/kg body weight/day, resulted in a significant dose-dependent increase in DNA damage in male mice germ cells following 6 months of exposure in the two highest dosage groups (1 and 10 μg/ml). While after 12 months of exposure, increases in damage were detected at doses as low as 0.01 μg/ml (0.001 mg/kg of body weight/day); where DNA damage was assessed using a Comet assay modified to detect adducts and γH2A.X expression, a marker of double-strand breaks (DSBs).The results of this study are the first to record that, the chronic exposure to AA at doses equivalent to human exposures; generates DNA damage in male germ cells of mice. In summary, DNA damage appeared to be both dose and time-dependent, and attributable to a range of genetic lesions triggered by either the production of free radicals via AA metabolism or the DNA-adducts formation by the AA metabolite, GA, where GA interacts with DNA in the testes/sperm or spermatid protamine resulting in the formation of DNA adducts that leads to male germ cell mutagenicity. In addition, a study by Ehlers et al. (2013) who investigated the effect of low and high doses of GA (1–0.0001 mM) on the expression of genes that are involved in the development of cancer using human ovarian and endometrial cancer cell lines and human primary hepatocytes; the authors reported high dose of GA (1 mM) can upregulate the expression level of the genes that have a role in oncogenesis process.

42.5 Other Toxic Effects of Acrylamide

42.5.1 Immunotoxicity

Although, the knowledge on the immunotoxic potential effect of AA is so limited, one study showed a significant decrease in the weight of spleen, thymus, and mesenteric lymph nodes of rats, after exposure to AA (Zaidi et al. 1994). In addition, Fang et al. (2014) reported a decrease in terminal body weight, thymus and spleen weights, and a count of lymphocytes in female BALB/c mice following exposure to AA. The researchers also informed a significant reduction in the percentage of natural killer cells and interleukin-6 level concentration. Kim et al. (2015) demonstrated that AA exposure could induce senescence in C57BL/6 male mice macrophages.

Besides, the researchers associated the AA-induced senescence to the production of ROS, activation of both p38 and JNK signaling pathways, and also, increased the expression level of p53 gene.

42.5.2 Hepatotoxicity

Although AA metabolism takes place in the liver, there were no reports of the hepatotoxicity of AA in humans. However, several investigations have recorded the harmful effect of dietary AA in the liver of experimental animals due to oxidative stress. A high dose of 25 mg/kg AA administrated for 21 days in experimental adult rats leads to significant depletion of liver GSH level, total antioxidant status, liver enzymes superoxide dismutase, catalase activities, while an increase in total oxidant status and malondialdehyde levels. Furthermore, the total cholesterol, triglyceride, and low-density lipoprotein cholesterol levels were reported to be increased, while high-density lipoprotein cholesterol was detected to be decreased (Ghorbel et al. 2017).

42.6 Carcinogenic Potential of Acrylamide in Human

Since the detection of AA in foods, numerous studies have conducted to understand the carcinogenic potential effect of AA in humans. Despite AA was shown to be carcinogenic in both male and female rodent models, several studies have illustrated no statistically significant association between dietary AA intake and different types of cancers in humans, such as pancreatic, prostate, breast, ovarian, and endometrial cancer (Pelucchi et al. 2017). Interestingly, Hogervorst et al. (2017) showed a significant interaction between the intake of AA and the single-nucleotide polymorphisms (SNPs) in CYP2E1 enzyme (rs915906 and rs2480258) with endometrial cancer risk. In addition, SNPs in the HSD3B1/B2 gene cluster (through effects on progesterone or androgens) with ovarian cancer risk were also detected, but confirmation is needed. These 2 *CYP2E1* SNPs located in the intronic region of the gene and thus don't affect the protein code, rather they may be in linkage disequilibrium with causative variants. In addition, there were some nominally statistically significant interactions with the SNPs in genes that contributed to the metabolism of AA, thus having a higher *a priori* probability of modifying the association between AA and cancer risk. Few studies reported a possible association between dietary intake of AA and the risk of cancer, e.g., dietary AA may cause an increase in the risk of cutaneous malignant melanoma and lymphatic malignancies (i.e., multiple myeloma and follicular lymphoma) incidence in men (Lipunova et al. 2016). Another study revealed that dietary intake of AA was associated with an increase in overall cancer mortality in elderly Chinese (Liu et al. 2017). In 1994, IARC classified AA as a potential carcinogen to humans (Group 2A) based on its carcinogenicity in rodents,

and this classification was endorsed by the WHO Consultation in 2002. A carcinogen in rodents and a suspected carcinogen in humans cause gene mutation and DNA damage (IARC 1994; Mojska et al. 2010). Some researchers reported that the increased risks of ovarian, endometrial, renal and esophageal cancers incidence were associated with AA consumption. While in 2011 a team of European researchers reported no convincing evidence of the relationship between AA exposure and tumor formation in humans. The current epidemiological and toxicological status of AA in a normal diet likely shows the negative impacts of causing pancreatic cancer with the highest cumulative exposure (Hogervorst et al. 2010). Also, it was shown that AA and GA act as biological agents for the initiation of the mutation process. The existence of genotoxicity occurs mainly through the formation of GA that is responsible for mutagenicity. The AA functions also as a Michael acceptor and forms adducts with thiol, hydroxyl or amino groups, exhibiting its toxicity at the nucleophilic centers in DNA (Carere 2006). A complete understanding of the associated risk helps in the effective management of AA; particularly there are no regulatory actions from authorities to control the AA consumption in food or the diet. Recently, various agencies adopted a minimization concept known as "as low as reasonably achievable" (ALARA) to control the consumption of AA via food.

The concentration of AA in potato chips was found to be extremely high compared to that of other food products, and this led to the development of research at an international level, with agencies such as WHO, FAO, and NCFST carrying out consistent research efforts on the potential risk of consumption of AA. But, the perception towards different food products is not consistent, because processing technologies differ concerning different populations. Although various studies have reported that the intake of AA can cause cancer, certain authors denied a consistent relationship between AA and cancer. A dose-response relationship has been implemented to assess the health benefits with the help of a margin ratio. AA was found to be genotoxic in vivo in somatic cells and germ cells; therefore AA has the potential to induce heritable damage at the gene and chromosome levels, where it is known to be metabolized to GA, a chemically reactive epoxide that forms DNA adducts. The findings that AA induces tumors both in rats and mice at several different sites are consistent with a genotoxic mode of action of the chemical. In conclusion, the Consultation endorsed the IARC classification Group 2A that AA is probably carcinogenic to humans. This genotoxic and carcinogenic substance is considered to be without a threshold for its action on DNA. For such a compound, it is generally recommended that exposures should be as low as reasonably achievable (ALARA) (Baskar and Aiswarya 2018). Another approach is to estimate carcinogenic risks which based on extensive epidemiological data that contain both accurate determinations of AA exposure and the tumor incidence in the exposed human population; however such data are rarely available. Only limited epidemiological data are available for AA. Since there was no knowledge of the significance of AA exposure from food, the influence of the background of this source of exposure was not evaluated. The application of new methods in biological research may be supportive in illustrative whether it is possible to establish a threshold for the genotoxicity of AA (WHO 2002).

42.7 Mitigation Strategies for Acrylamide Formation

According to the American Cancer Society, it's not yet clear if the levels of AA in foods raise cancer risk, but if you're concerned, there are some things you can do to lower your AA exposure. In general, many strategies were shown to be able to inhibit or mitigate AA formation, both in model systems and actual food systems. These strategies include modification of pH, decreasing the time and heating temperature, choosing raw materials with fewer precursors, adding the different exogenous additives, and process technology interventions (such as; acids, amino acids, hydrogen carbonates, proteins, and antioxidants). For instance, treatment of the wheat-based dough with asparaginase that was produced from *Cladosporium* sp. (to convert the precursor asparagine to aspartic acid), was able to lessen 97 and 73% of AA formation in the crust and crumb regions of the bread. Likewise, a 90% reduction in the content of AA will be reached after using a combination of asparaginase treatment and blanching of potato slices. In a separate study, the addition of asparaginase was also reported to reduce the AA content in the chilled French fries up to 90% without affecting the final taste of the product (Kumar et al. 2018). Though treatment with asparaginase is a promising strategy for the reduction of AA content, it is rather expansive compared with other strategies. Moreover, it was detected that the addition of amino acids or protein-rich substances could reduce the AA content in foodstuffs. Amino acids such as glycine, cysteine, methionine, glutathione, and lysine can reduce the formation of AA and its elimination kinetics was assessed in numerous studies. When cysteine and methionine were added to cracker and potato dough, AA formation was found to be decreased by 50%. On the other hand, Flückiger and Salih (2006) didn't find such results when studied the effect of cysteine on AA formation in crispbread. Addition of antioxidants to foods has been detected to influence the Maillard reaction, which results in the formation of AA. Antioxidants that are present in the rosemary extracts, bamboo leaves, and green tea extract could effectively decrease the presence of AA in different heated foods (Krishnakumar and Visvanathan 2014).

The strategies developed so far to diminish AA formation studied in lab conditions, which may not be suitable for commercial process. Therefore, further research work in the laboratory is necessary to explore different expectations under industrial conditions. Reduction of AA in food products while protecting other quality aspects and lowering dietary AA exposure remains a major challenge. Several studies used antioxidants as additives to prevent the formation of AA and the inhibition of AA formation was in correlation with antioxidant activity of the additives. One study found that AA formation was significantly inhibited during microwave heat processing after the addition of flavonoids (Cheng et al. 2015). As well, phenolic antioxidants demonstrated to have an inhibiting effect on AA formation, with the most significant reductions (≈60%) observed for caffeoylquinic acids (Constantinou and Koutsidis 2016). Alternatively, the addition of natural herbal extracts also has an inhibitory effect on AA formation. For example, the addition of antioxidants of bamboo leaves (AOB) and acylated AOB were able to lessen the formation of AA in

fried potato crisps ranging from 30.7% to 46.9% (Ma et al. 2015). Identically, the addition of green tea extract into burgers and nuggets coating were able to reduce the formation of AA (Soncu and Kolsarici 2017) (Table 42.1). Combination of lysine with antioxidants might be tried as a mitigation strategy where lysine is an essential amino acid that works to prevent the invasion of healthy tissues by tumorigenic cells (Hashim et al. 2011), particularly when combined with ascorbic acid (vitamin C). While the combination with amino acid, glycine, is not recommended because it found to enhance the growth of normal and cancerous cells (Weinberg et al. 2016).

Table 42.1 Some mitigation strategies for acrylamide formation reduction in different foodstuffs. (Baskar and Aiswarya 2018)

Types of food matrix	Additives added	Conditions	Remarks
French fries	NaCl and SAPP	The sample was cut, washed and fried immediately.	71% and 95% reduction without change in the sensorial properties.
	$CaCl_2$	Dipped in distilled water at 37 °C for 60 min.	93% acrylamide reduction
	Asparaginase	Blanched in distilled water at 75 °C for 10 min.	62% acrylamide reduction
Potato slices	Asparaginase	Frying at 170 °C for 15 min.	82% reduction
Crisps	Lysine and Glycine	Soaked in water at 65 °C for 5 min.	94% reduction
	Citric and acetic acid	Blanched in water at 70 °C for 3 min.	32% reduction
	Antioxidants	–	50% reduction
Cereals	Legume proteins	–	Reduces because of reaction between SH and NH2
Wheat crackers	Asparaginase	–	70% reduction without any change
Biscuits and crust systems	Chitosan	–	81% reduction
Gingerbreads	Sodium hydrogen carbonate	–	70% reduction
Crackers	Cysteine and methionine	–	50% reduction
Fried batter	Chitosan	–	59 ± 6% reduction

42.8 Conclusion

According to the detailed explanations that were shown through this chapter about the risk assessment of human carcinogenicity of acrylamide (AA) in food, it can be concluded that:

1. Everyone (from baby to elder) is exposed through diet to AA, with variant exposure ranges, that could be formed by the high-temperature cooking process via different mechanisms and thought to be a probable human carcinogen that has an impact on cancer risks.
2. Acrylamide (AA) could lead to cancer through enhancing alterations in the redox status of the cells and causing DNA damage by its genotoxic metabolite, glycidamide (GA).
3. Although AA was studied to elevate the probability of carcinogenicity in humans, the only limited number of foods-worldwide-have been analyzed for AA content, and no data are available for foods consumed in many countries particularly in developing countries. So that it must take into consideration, the labeling of each food with the detected AA concentration so the exposure limit could be calculated to avoid the risk of overdose. Also, analysis of blood and urinary biomarkers of AA exposure needs to be calibrated and their correlation to dietary intake should be investigated.
4. Despite there are many mitigation strategies that could be applied in the food industry to reduce the concentration of AA in consumed foods, further safe strategies are still required but want to be tried first in the research lab before their use for humans.

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