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REVIEW ARTICLE

Acrylamide in bread: a review on formation, health risk assessment, and determination by analytical techniques

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Abstract

Acrylamide is a water-soluble toxicant found in high-protein and carbohydrate-containing foods exposed to high temperature like bread as the staple foodstuff. This toxicant is mainly formed via Maillard reaction. The potential adverse effects of acrylamide especially possible carcinogenicity in human through dietary exposure necessitate its monitoring. Regarding the existence of its precursors in wheat bread formulation as well as extreme consumption of bread by most population and diversity of bread types, its acrylamide level needs to be investigated. The indicative value for acrylamide in wheat bread is set at 80 μg/kg. Consequently, its determination using liquid chromatography–tandem mass spectrometry (LC-MS/MS), gas chromatography–mass spectrometry (GC-MS), or capillary electrophoresis can be helpful considering both the risk assessment and quality control aspects. In this respect, methods based on LC-MS/MS show good recovery and within laboratory repeatability with a limit of detection of 3–20 μg/kg and limit of quantification of 10–50 μg/kg which is suitable for the immediate requirements for food product monitoring and calculation of consumer exposure.

Keywords Acrylamide · Maillard · Bread · Risk assessment · High-performance liquid chromatography · Gas chromatography

Introduction

Bread has been considered as the main food constituent and basic food resource for thousands of years providing essential components like proteins, carbohydrates, fibers, and vitamins (Mollakhalili Meybodi et al. 2015). In this regard, bread directly affects human health. Although the baking process is effective on making the bread more palatable via improvement of the color, flavor, taste, and texture, some hazardous compounds like acrylamide may also be formed (Meybodi et al. 2019b; Wang et al. 2017). Acrylamide or acrylic amide (2-propenamide/CH_2=CHC(O)NH_2) is a white, low molecular weight crystalline solid which is highly water soluble. It is one of the neurotoxic and carcinogenic substances found in foods with low moisture content exposed to temperatures higher than 120 °C like bread (Keramat et al. 2011a). The toxicological characteristics of acrylamide including genotoxicity, neurotoxicity, carcinogenicity, and reproductive toxicity have been well investigated. It has been recognized as a probable carcinogen (group 2A) by the International Agency for Research on Cancer which is also "probably carcinogenic to humans" (Svensson et al. 2003). The indicative value for acrylamide in wheat bread and soft breads other than wheat-based ones are set at 80 and 150 μg/kg, respectively, according to scientific opinion on acrylamide in foods as reported by European Food Safety Authorization (EFSA 2015). Acrylamide is an electrophilic α, β-unsaturated carbonyl compound which is prone to react with nucleophilic groups on biological molecules. In vivo, acrylamide is converted to an electrophilic epoxide glycidamide by cytochrome P450 which its DNA binding is considered as the genotoxicity cause of acrylamide (Crawford

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et al. 2019b). Various toxicological investigations suggested that acrylamide inhalation provides the paralysis of the cerebrospinal system and irritates the eyes and skin (Zhang et al. 2005). Acrylamide, which is transferred to different parts of the body via the blood stream, is found in many tissues and parts of the body (Hogervorst et al. 2007). The mechanisms by which acrylamide is formed is necessary to be investigated thoroughly to decrease its potential detrimental effects. Acrylamide in breads is mainly formed through Maillard reaction of the asparagine and reducing sugars, as the main precursors (Nasiri Esfahani et al. 2017). In addition, other routes have been also proposed for acrylamide formation (Granvogl and Schieberle 2006; Hamzalıoğlu et al. 2019; Liyanage 2019). However, the quantity of precursors involved in acrylamide formation, the processing methods, and conditions may also affect the formation of acrylamide.

As the long-term consumption of acrylamide may detrimentally affect human health, its analysis is of great importance regarding both its accurate risk assessment and also the quality control to minimize its formation. Consequently, the development of an appropriate and rapid responsive method is necessary to be achieved. Different methods have been reported to quantify the acrylamide and its derived metabolites, such as gas chromatography (GC), high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), and ELISA. These methods differ greatly according to their sensitivity, complexity of sample preparation, ease of use, efficiency, and cost (Longhua et al. 2012). Considering the importance of bread as a staple foodstuff, its suitable condition for formation of acrylamide, and the health risk of acrylamide as a carcinogenic compound to humans, the aim of this review article was to investigate the formation, health risk assessment, and determination of acrylamide in breads.

Acrylamide formation

Several routes have been suggested for acrylamide formation in bread that are summarized in Fig. 1. It is pointed out that acrylamide is typically formed in bread by the reaction between free amino acids and reducing sugars during bread cooking. In other words, combination between amino groups of free amino acids and carbonyl compounds of reducing sugars (mainly glucose and fructose) during Maillard reaction generates considerable amounts of acrylamide (Nasiri Esfahani et al. 2017). Maillard as a non-enzymatic reaction is involved in bread's desirable color and aroma, along with the formation of beneficial and damaging compounds (Meybodi et al. 2019a). At the first step of the reaction, Schiff bases are formed by the interaction between carbonyl and amino groups. The Schiff bases rearrange to Amadori or Heyns products that subsequently produce aroma and color with potential health benefits in the next stages (Ledl and Schleicher 1990). Furthermore, the Schiff bases may be decarboxylated or hydrolyzed to form azomethine ylide and 3-aminopropionamide (3-APA), respectively. Thereafter, acrylamide may be formed via deamination of 3-APA or directly by azomethine ylide (Granvogl and Schieberle 2006; Hidalgo et al. 2010).

The main precursors of acrylamide are free asparagine and reducing sugars which are affected by crop's type and cultivar and harvest season (Curtis and Halford 2016). Studies related to the free asparagine content of different wheat species and cultivar in the same environmental conditions indicated that rye, einkorn, emmer, wheat, and spelt had higher content of free asparagine, respectively. Differences also have been observed in different cultivars (Stockmann et al. 2018). Generally, cultivars with higher content of free asparagine resulted in higher acrylamide content in the final products. Higher content of protein and amino acid in crops cultivated with desirable light and temperature as well as nitrogen fertilization led to a higher acrylamide concentration in final products. Grain cultivated under sulfur-deficient conditions will also provide high quantity of asparagine which makes it more prone to form acrylamide during thermal processing (Curtis and Halford 2016). Great rainfall almost before harvesting and storing the crops under unsuitable conditions (high humidity and temperature) have been proposed to increase the activity of enzymes involved in acrylamide formation and grain sprouting (Stockmann et al. 2019). Damaged starch resulted in the formation of higher acrylamide in final products due to an increase in availability of reducing sugar (Wang et al. 2017). Overall, breads with higher fructose content had greater amount of acrylamide (Shen et al. 2019). The flour type is supposed to be significantly effective on acrylamide formation as the quantity of acrylamide is higher in breads provided by high extraction rate flours compared to flour having lower nutritional value (Namir et al. 2018). High extraction rate flours contain higher levels of fibers and reducing sugars leading to greater affinity to be involved in Millard reaction and thus higher level of acrylamide.

In the case of processing factors, high temperature and low water activity are considered as the major effective factors in acrylamide formation (Keramat et al. 2011a). The bread's crumb and crust are separately influenced by the baking process. While the crust is undergoing high temperature (more than 200 °C) and low water activity (a_w), the crumb is exposed to lower temperature and higher a_w. Consequently, high amount of acrylamide is accumulated in crispy and brown crust of bread, while the crumb contains no or very low levels of acrylamide (Liu et al. 2018).

Acrylamide mitigation

It is known that several factors influence the acrylamide mitigation in bread that can be categorized as bread ingredients

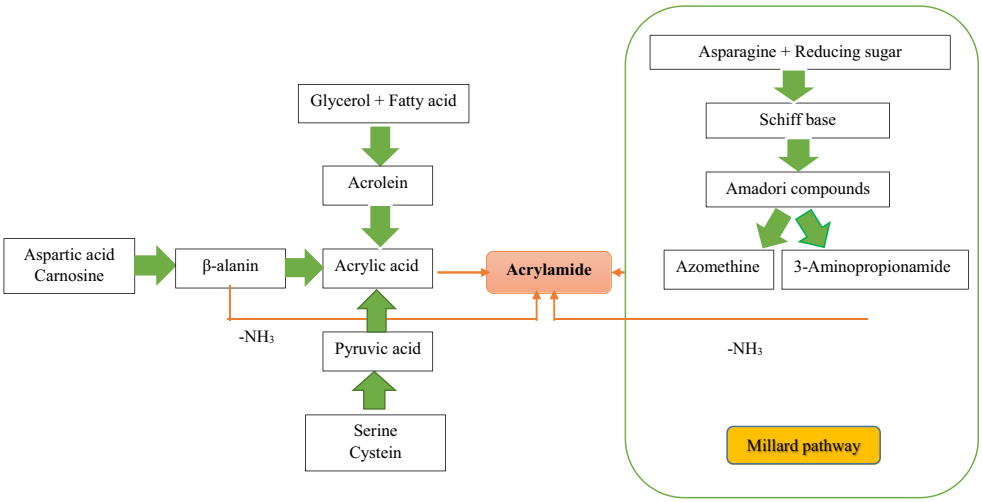

Fig. 1 Potential mechanisms for acrylamide formation in bread

and processing conditions. Changes in these factors may reduce acrylamide formation or increase acrylamide removal and, consequently, final bread with lower or no acrylamide content.

Bread ingredients

The main precursors of acrylamide are free asparagine and reducing sugars which are affected by crop's type and cultivar, harvest season, and storage condition. Accordingly, its reduction or dilution is considered as a prevalent strategy to decrease acrylamide formation in bread. Asparagine could be consumed in bread formulation by inclusion of asparaginase, yeast, or mixed microorganisms of sourdough (Xu et al. 2016). Asparaginase as an enzyme commonly produced from *Aspergillus niger* (Vala et al. 2018), *Aspergillus oryzae* (Dias et al. 2019), and bacterial sources, especially probiotics such as *Bacillus* sp. strain M6 (Ray et al. 2019), is able to decrease acrylamide formation by transforming asparagine to aspartic acid and ammonia. The capability of pea-originated asparaginase in decreasing acrylamide has been investigated in different types of breads. Results indicated 57% and 68% reduction in acrylamide levels in wheat bran and whole-grain wheat breads, respectively (Tuncel et al. 2010).

The addition of complexing agents like polyvalent cations is considered as another strategy to decrease acrylamide formation through binding with asparagine and preventing formation of Schiff bases as intermediate compounds (Pedreschi et al. 2010). Various studies indicated the remarkable reduction in acrylamide content through polymerization by appropriate levels of NaCl, while acrylamide value incremented at higher NaCl concentration that was ascribed to yeast growth inhibition (Frigon and Liu 2016). Adding suitable antioxidants at appropriate concentrations is also effective in decreasing the acrylamide formation. However, their exact effects are not comprehended yet. Antioxidants are suitable for reducing asparagine by its precipitation, trap carbonyl compounds, and reacting with Maillard intermediates. They can also destroy acrylamide by Michael addition-type reactions or other destructive reactions (Jin et al. 2013). The application of (−)-epigallocatechin gallate, a type of green tea antioxidant, in bread has been reported to decrease acrylamide formation by 37% (Fu et al. 2018). In another study, the effect of addition of buckwheat extract as a source of antioxidants on acrylamide formation in bread showed a reducing effect as well as an improvement in bread color (Jing et al. 2019). Garlic powder at level of 0.05 g showed a similar effect on acrylamide formation (Li et al. 2016).

The removal of acrylamide forming accelerator like ammonium salts is another strategy to decrease acrylamide formation in bread (Curtis and Halford 2016). Ammonium hydrogen carbonate which is used as a baking agent to generate gas during bread baking has boosted the acrylamide formation. In this regard, its replacement with alternatives, like sodium hydrogen carbonate, without undesirable effects on final products is appealing (Komprda et al. 2017). The addition of amino acids like glycine which reduce acrylamide formation through competition with asparagine in Maillard reaction or inactive the acrylamide via Michael addition-type reactions is also reported to be effective in reducing acrylamide formation in bread (Zhu et al. 2016).

Processing factors

Nearly most strategies suggested to decrease the acrylamide formation are focused on the processing steps. Substantial processing factors in bread baking are water activity, fermentation (acidity, inoculated microorganisms, and consequently their metabolites), and baking (time and temperature). High temperature and low water activity during baking that are required for desired crust properties are responsible for acrylamide formation. As the dark brown color and crispy texture

of the crust are important parameters in sensory acceptance of bread, using steam during final 5 min of baking or infrared radiation for bread baking have been reported to lower the acrylamide level in the final products while maintaining the bread color (Keramat et al. 2011a). It seems that baking at lower temperature and prolonged time will also be efficient (Przygodzka et al. 2015). As acrylamide is formed mainly in the crust, strategies including spraying the glycine solution on the surface of the dough (Fink et al. 2006), brushing aqueous cysteine solution to dough before baking (Claus et al. 2008b), combining addition of glycine and corn starch, and corn or potato starch coating (Liu et al. 2018) have also been considered effective.

Consumption of asparagine, as a source of nitrogen, along with microorganism inoculation (yeast or sourdough) could also decrease the acrylamide level. Prolonged fermentation process with yeasts results in higher asparagine utilization (Sadd et al. 2008). Sourdough fermentation is more effective in decreasing acrylamide formation as it reduces the pH (4.4–4.8) more and degrades the main precursors of acrylamide (Bartkiene et al. 2017b). The most found species in sourdoughs consist of *Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus sanfranciscensis*, and *Lactobacillus acidophilus* (Viiard et al. 2016). Several studies were performed regarding utilization of lactic acid bacteria (LAB) sourdough in bread making. Some studies dealing with the reducing effect of LAB on acrylamide formation are summarized in Table 1. Using probiotic bacteria is another approach in mitigation of toxins (Arab et al. 2019, 2012; Yousefi et al. 2019). In this respect, probiotics are able to decrease acrylamide formation by producing asperginase and removing produced acrylamide via cell wall peptidoglycan (Khorshidian et al. 2020).

Risk assessment of acrylamide

Risk assessment is a scientifically based process for assessing whether a contaminant like acrylamide causes a risk through consumption of a specific food such as bread in a given population (Eslamizad et al. 2019; Yousefi et al. 2018). The program steps are shown in Fig. 2.

Acrylamide is known as a possible human carcinogen and is proved to create several tumors in experimental animals (Jesus et al. 2018). At the beginning, researchers from Sweden have reported that acrylamide can be formed during heat processing of foods rich in carbohydrates and proteins like breads and fried potato products as a by-product of Maillard reaction (Tareke et al. 2002). As bread is a staple food in human diet all over the world and is a good source of carbohydrates and proteins (Diana et al. 2014), acrylamide occurrence should be considered as a health concern in this important food. In the following sections, the risk assessment process of acrylamide through bread consumption is explained step by step.

Hazard identification

The adverse health effects via exposing to acrylamide is necessary to be identified for planning to avoid and/or mitigate its carcinogenicity, neurotoxicity, genotoxicity, and reproductive toxicity (Negoiță et al. 2014). Therefore, it is necessary to investigate the toxicokinetics (absorption, distribution, metabolism, and excretion) and toxicodynamics (main endpoints in target organs) of acrylamide in hazard identification step based on animal studies and occupational exposure in humans (Mousavi Khaneghah et al. 2020).

Toxicokinetics

Acrylamide is absorbed via ingestion, inhalation, or dermal contact in the animal and human body. It can distribute rapidly in all organs (Virk-Baker et al. 2014) and is observed in several parts and tissues within the body especially liver, brain, thymus, kidney, and heart (Nematollahi et al. 2019; Norouzi et al. 2018). However, no evidence is provided for acrylamide accumulation in any tissues. After absorption and distribution, it is oxidized by detoxification enzymes mainly cytochrome P450 (CYP450) 2E1 in the liver and is transformed to a reactive epoxide-glycidamide which is a genotoxic agent, more toxic than acrylamide (Gökmen 2015). Both acrylamide and glycidamide can undergo the second detoxification phase and conjugate with glutathione by action of glutathione-*S*-transferase enzymes to form less toxic cysteine metabolites (mercapturic acid derivatives) (Pedreschi et al. 2014). Furthermore, glycidamide can also be hydrolyzed to form the non-toxic glyceramide (2,3-dihydroxypropanamide) and then 2,3-dihydroxypropionic acid. Acrylamide can pass the placenta and is also found in breast milk (Lineback et al. 2012). Finally, the intact acrylamide and its epoxide metabolites (glycidamide) as well as its mercapturic acid derivatives are eliminated via urine (Gökmen 2015; Jesus et al. 2018).

Toxicodynamics

Both acrylamide and glycidamide could react with DNA, hemoglobin, and proteins to form special adducts which might damage cells and are considered as biomarkers for acrylamide after exposure (Xu et al. 2014). It is revealed that acrylamide and glycidamide have shown genotoxicity, teratogenicity (Crawford et al. 2019a), neurotoxicity, carcinogenicity, and reproductive toxicity effects after accidental and occupational as well as dietary exposure in human and animal studies (Motaghi et al. 2012; Pundir et al. 2019).

Table 1 Various studies about the effect of LAB sourdough on acrylamide formation in bread

LAB	Type of bread	Preparation method	Results	References
P. acidilactici, *L. plantarum*, and *L. curvatus*	Mixed rye–wheat bread	5–15% sourdough was added to the dough	All LABs resulted in lower acrylamide levels in final products compared to control samples. *L. plantarum* was the most effective bacteria in decreasing acrylamide content	(Bartkiene et al. 2017a)
Pediococcus acidilactici KTU05-7, *Pediococcus pentosaceus KTU05-8*, *P. pentosaceus KTU05-9*, *Lactobacillus sakei KTU05-6*, *P. pentosaceus KTU05-10*	Wheat bread with fermented Jerusalem artichoke tubers	Jerusalem artichoke tubers were fermented with LABs	Higher amylase activity of LABs and pH of the sourdough resulted in lower acrylamide forming in bread	(Bartkiene et al. 2013)
Lactobacillus plantarum LUHS135	Mixed rye–wheat bread	Rye, wheat, barley, and oat sourdoughs were produced and 5, 10, 15, and 20% of them were added to the dough separately	The best sourdoughs for reducing acrylamide formation were wheat sourdough (5 and 10%), barley sourdough (5, 15, and 20%), and oat sourdough (5 and 15%)	(Bartkiene et al. 2017b)
P. pentosaceus LUHS183, *P. acidilactici LUHS29*, *Lactobacillus paracasei LUHS244*, *Lactobacillus brevis LUHS173*, *Lactobacillus plantarum LUHS135*, and *Leuconostoc mesenteroides LUHS242*	Wheat bread	10, 15, and 20% sourdough were added to the dough	Applying 20% sourdough resulted in lower acrylamide content	(Bartkiene et al. 2018)
Lactobacillus casei-casei and *Lactobacillus reuteri*	Sangak (whole wheat flour bread) and bread roll (white wheat flour bread)	13 g sourdough was added to bread's formula	Sangak breads showed lower acrylamide contents compared to bread roll. Breads fermented by *L. casei-casei* showed lowest acrylamide levels	(Dastmalchi et al. 2016)
Lactobacillus casei and *Lactobacillus rhamnosus*	Sangak and Bulk bread (white wheat flour bread	13 g sourdough was added to bread's formula.	LAB sourdough decreased acrylamide in both type of bread. Acrylamide levels of Sangak bread were lower.	(Dastmalchi and Razavi 2016)
Lactobacillus plantarum PTCC 1896, *L. sakei DSM 20017*, *L. rhamnosus DSM 20021*, and *L. delbrueckii DSM 20081*	Whole-wheat bread	75 g sourdough was added to bread's formula	Breads fermented by sourdough and yeast had lower level of acrylamide compared to bread fermented by yeast only *L. rhamnosus* was the most effective strain in reducing acrylamide	(Nasiri Esfahani et al. 2017)

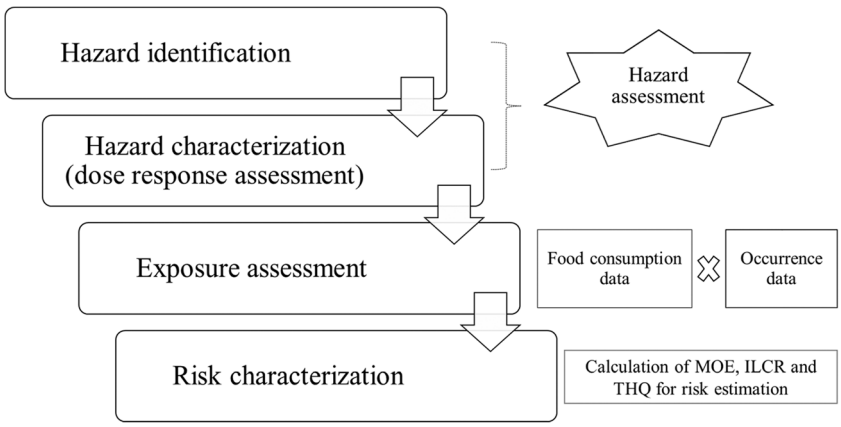

Fig. 2 Steps involving in risk assessment paradigm

Hazard characterization

As acrylamide is a genotoxic and carcinogenic compound (Dybing and Sanner 2003), there is no threshold in its dose–response relationship (non-threshold compound). NOAEL (no observed adverse effect level) for neurotoxicity (based on morphological alterations in the rat nervous system) and reproductive effects of acrylamide are reported as 200–500 and 2000–5000 μg/kg BW (body weight)/day, respectively, by JECFA (Joint FAO/WHO Expert Committee on Food Additives) (Claus et al. 2008a). Turning to carcinogenic toxicity of acrylamide, $BMDL_{10}$ (benchmark dose lower confidence limits for a 10% extra risk) of Harderian and mammary gland tumors in rodents are stated as 180 in mice and 310 μg/kg BW/day in rat, respectively, by JECFA (Pedreschi et al. 2014; Sirot et al. 2012). The European Food Safety Authority (EFSA) reported the levels of 170 and 430 μg/kg BW/day for carcinogenic and neurotoxic effects in rats, respectively. For neurotoxicity and carcinogenicity effects of acrylamide, the TDI (tolerable daily intake) has been set as 40 and 2.6 μg/kg BW/day (Pundir et al. 2019).

Exposure assessment

Exposure assessment is performed for evaluating whether acrylamide is a risk for the specific population or not (Cengiz and Gündüz 2013). For estimation of acrylamide exposure via food consumption, two types of data are required including occurrence data (acrylamide concentration in bread obtained by analytical instruments) and consumption data (the intake rate of food products by a given population obtained by questionnaires) (Nematollahi et al. 2020b). Subsequently, the acrylamide exposure via food consumption is calculated by multiplying these two data and then divided by the mean body weight of the population and expressed as milligrams or micrograms of acrylamide/kilogram BW/day (Boyacı Gündüz and Cengiz 2015). Acrylamide exposure by a certain food product is influenced by both its acrylamide content and its intake ratio (Cengiz and Gündüz 2013). In this regard, a food item may contain low acrylamide content, but can have a great contribution rate to dietary exposure if its consumption rate is high (Nematollahi et al. 2020a). For example, bread, despite having low acrylamide level, is considered as an essential source of acrylamide exposure owing to its relatively high consumption in many populations like Iranian, German, and Romanian peoples which consume 320, 240, and 300 g breads per day on average, respectively (Boyacı Gündüz and Cengiz 2015; Claus et al. 2008a; Eslamizad et al. 2019). Likewise, some foods such as ginger bread in spite of having high acrylamide concentration is not a main contributor of dietary acrylamide exposure due to its low consumption rate (Lineback and Jones 2011).

The consumption data are obtained by food frequency questionnaire (FFQ), 24-h dietary recall in addition to food records and are usually expressed as grams/day (Zajac et al. 2013).

There are three different methods used in exposure estimation which are deterministic (qualitative or point estimates), semi-probabilistic (semi-quantitative), and probabilistic methods (quantitative done by Monte Carlo simulation) (Gökmen 2015; Zajac et al. 2013). The most precious method is probabilistic approach in which a distribution of acrylamide content is pooled with a distribution of intake rates, giving rise to a distribution of dietary acrylamide exposure that is largely respected more demonstrative of the real exposure (Mills et al. 2008).

The acrylamide levels in the studied bread samples are very different due to variations in formulation and bread-baking methods between different cultures, the number and nature of the bread samples, and the approach used for the estimation in addition to the nature of consumption investigation (Claeys et al. 2010; Nematollahi et al. 2019). Acrylamide concentrations in different types of breads have been explored by several countries (Crawford et al. 2019b; Jing et al. 2019; Longhua et al. 2012; Marconi et al. 2010). JECFA announced the average levels of acrylamide in crispy breads in a wide range of 87–459 μg/kg, while EFSA reported an average level of 223 μg/kg in soft breads (EFSA 2015).

In addition to the differences in acrylamide concentration in different countries, their consumption habits are extremely diverse (EFSA 2015). Most studies have reported the dietary exposure to acrylamide for all consumed foods not only for bread consumption. For instance, FAO/WHO (Food and Agriculture Organization/World Health Organization) has established the acrylamide dietary exposure of 0.3–0.8 μg/kg BW/day (Altunay et al. 2016). In addition, JECFA has reported average and high (high percentiles—95th–97.5th) dietary acrylamide exposure of 1 and 4 μg/kg/day, respectively (Negoiță et al. 2014). However, in a Turkish study, the acrylamide dietary exposure through bread is estimated as 0.58 μg/kg BW/day (Cengiz and Gündüz 2013). It is worthwhile to mention that the dietary acrylamide exposure of children is two to three times further than adults due to their lower body weight (Mojska et al. 2010). The estimations reported by JECFA revealed that the general foods contributing to acrylamide dietary exposure are fried potato products (like French fries and crisps; Kamankesh et al. 2020) and bakery products (mainly breads, pastry, cookies, and biscuits). However, EFSA mentioned bread, coffee, and potatoes as the main contributors to the acrylamide exposure (Eslamizad et al. 2019; Virk-Baker et al. 2014). Given the differences in cooking and eating traditions and habits between different countries, the main contributor foods in dietary acrylamide exposure might be different (Virk-Baker et al. 2014).

Risk characterization

The final and ultimate part of acrylamide risk assessment procedure is risk characterization in which all of the information collected from the hazard assessment and exposure assessment steps are put together in order to estimate the real risk through acrylamide exposure via bread (Pedreschi et al. 2014). In fact, in this step, the dose–response correlation for different adverse endpoints are compared with the calculated dietary exposure to acrylamide. This step acts as the intermediate of the risk assessors and risk managers in risk analysis paradigm (Xu et al. 2014). It should bear in mind that the risk estimation by different approaches is performed for calculation of carcinogenicity and non-carcinogenicity risk related to acrylamide exposure through bread consumption (Zajac et al. 2013). These approaches are explained in the following:

Margin of exposure (MoE): MoE, used by JECFA and EFSA, is a common output in the risk characterization step of contaminants that are both carcinogenic and genotoxic which is based on experimental animals' data (Zajac et al. 2013). To calculate MoE (dimensionless) for dietary exposure to acrylamide, the $BMDL_{10}$ obtained for different induced tumors in rats and mice (from dose–response assessment step) is divided by the dietary exposure of a given population achieved from exposure assessment step.

The lower the MoE, the higher the risk of dietary exposure to acrylamide (Claeys et al. 2010). The EFSA endorsed that the MoE of 10,000 or smaller would be of high concern for public health, in terms of carcinogenicity effects, and should be counted as a high priority related to risk management activities (Altissimi et al. 2017; Shahrbabki et al. 2018). Thus, these very low estimated MoE show high health risk in the population related to dietary exposure to acrylamide (Gökmen 2015). In the same manner, EFSA used $BMDL_{10}$ of 170 and 430 μg/kg BW/day for incidence of neoplastic and non-neoplastic effects (neurotoxicity) in rodents (Kafouris et al. 2018). The EFSA stated that, for non-carcinogenic effect, a MoE below125 indicates concern of health risk for the population. The calculated MoEs by EFSA were 425 for adults (average consumers) and 50 for toddlers (high consumers) which shows high health concern, while for neurotoxicity, the MOEs were 1075 and 126, respectively, which reveals no concern (EFSA 2015). Furthermore, it is obvious that the risk of carcinogenicity and neurotoxicity through acrylamide dietary exposure is high and low (negligible), respectively (Branciari et al. 2019; Kafouris et al. 2018).

Incremental lifetime cancer risk (ILCR): Another applicable output of the risk characterization is estimation of the risk by ILCR calculation which is representative of cancer risk related to chronic dietary exposure to acrylamide (Shahrbabki et al. 2018) as calculated by Eq. 1:

$$\mathrm{ILCR} = (\mathrm{E} \times \mathrm{EF} \times \mathrm{ED} \times \mathrm{SF} \times \mathrm{CF} \times \mathrm{ADAF})/\mathrm{AT} \quad (1)$$

where ILCR (dimensionless) = the additional possibility of cancer over human lifetime, E = dietary exposure (μg/kg BW/day), EF = exposure frequency (days/year), ED = exposure duration (years), SF = oral cancer slope factor of acrylamide (0.5 per (mg/kg)/day), CF = correction factor (10^{-3} mg/μg), ADAF = age-dependent adjustment factor (dimensionless): for children is 3 and for adults is 1 (US EPA 2010), and AT = average time for carcinogens (25,550 days).

The permissible ILCR established by the US Environmental Protection Agency (US EPA) is 1×10^{-5}, which means that the risk of cancer is 1 person in each 10,000 exposed population. For instance, Eslamizad et al. (2019) reported the ILCR for acrylamide via bread consumption is greater than the permitted ILCR (1×10^{-5}), which is representative of elevated cancer risk which is high only for consumption of bread in Iran (Eslamizad et al. 2019).

Target hazard quotient (THQ): The THQ is another parameter for assessing the risk of non-carcinogenicity through acrylamide exposure. It is dimensionless and calculated with dividing dietary exposure by the oral reference dose (safe dose) for acrylamide (2 μg/kg BW/day) obtained from dose-response assessment (US EPA, 2010). If the value of estimated THQ is higher than 1, it means that the non-carcinogenicity is high in viewpoint of health concern. The greater the THQ value, the greater the neurotoxicity effect (Oroian et al. 2015). In the study of Eslamizad et al. (2019), the estimated THQ via bread consumption in Iran was lower than 1, which represented negligible and non-considerable neurotoxicity risk through bread intake (Eslamizad et al. 2019). It is worth mentioning that the risk of carcinogenicity through acrylamide dietary exposure is high while the risk of neurotoxicity (non-carcinogenic risk) is low due to the presence of small amounts of acrylamide in food for inducing neurotoxic symptoms in people (Branciari et al. 2019).

Uncertainties in acrylamide risk assessment

There are several uncertainties in risk assessment procedure. For instance, in this process, the dose–response's information (such as $BMDL_{10}$) obtained from experimental animals is used which cause uncertainties owing to extrapolating these data to human with considerable difference with rodents (Sirot et al. 2012). Furthermore, it was observed that acrylamide concentration in various breads and between the same types of bread were significantly different which affected the estimated risks (Kafouris et al. 2018). The reasons for these differences may be due to differences in type and variety of cereal grains, processing methods (Nematollahi et al. 2019), number of investigated samples, analytical techniques as well as number and properties of the studied population (for achieving body weight and consumption data) and statistical calculations (deterministic, semi-probabilistic, and

probabilistic methods). In addition, there is lack of information considering the effect of food on acrylamide bioavailability which also causes uncertainty in risk assessment (Konings et al. 2003). All of the noted uncertainties might underestimate or overestimate the outputs of risk assessment procedure such as the true risk level. For example, considering the use of statistical methods, it is proved that probabilistic approach via Monte Carlo simulation (by high iteration for concentration and consumption data) is the most promising approach due to quantifying the uncertainties related to estimated health risks which is not possible in deterministic approaches (Shahrbabki et al. 2018). It is worthwhile to mention that some of these uncertainties could be reduced by analyzing more food samples, obtaining food consumption data by at least two types of questionnaires (such as FFQ and food records), and using a probabilistic model (Arisseto et al. 2009).

Risk management

Although there is no determined safe limit for acrylamide in any foods, the safe limits considering its neurotoxicity effects have been established by EPA in water as 0.5 μg/L (US EPA 2010). On the other hand, Germany acquired an acrylamide reduction procedure, as a risk management paradigm, to persuade producers to reduce acrylamide content to the possible levels, which considers as low as reasonably achievable (ALARA approach) (Wyka et al. 2015). In this approach, while the acrylamide level in foods be higher than the signal value, the manufacturers were announced for its minimization by improving the formulation and processing (Branciari et al. 2019). In this view, the EC has established indicative values for acrylamide concentrations in different food products. These levels are not safety limits, but represent the need to investigate the possible reasons for its higher level compared to the indicative values (Lineback et al. 2012). This regulation has set the benchmark levels of 800, 350, 100, and 50 μg/kg for ginger bread, crisp bread, soft bread (except wheat), and soft bread (wheat-based), respectively (Cengiz and Gündüz 2013). Thus, the acrylamide content in breads should be reduced in order to decrease the dietary exposure to acrylamide in the whole population especially in the case of children. The main strategies investigated to decrease the acrylamide formation in breads are mentioned in previous sections. Given that acrylamide could cause a health risk for human just regarding its chronic exposure and also the shortcoming of human toxicological and epidemiological evidences to show the possibility of dietary exposure to acrylamide for induction of adverse effects in human health especially cancer, more studies are needed to investigate this field. It is reported that by reducing formation of acrylamide in breads, the dietary exposure in the US population decreased from 0.43 to 0.34 μg/kg BW/day (Mucci and Wilson 2008).

Analytical methods for determination of acrylamide in bread

Several analytical procedures have been developed for quantifying the acrylamide in foodstuffs since the discovery of this compound in human diet (Keramat et al. 2011b). These procedures must be fast, convenient, and reliable with low cost (Kim et al. 2011). The most frequently used techniques for acrylamide quantification in food products are high-performance liquid chromatography (HPLC), gas chromatography (GC), and capillary electrophoresis (CE) (Tekkeli et al. 2012). Most organizations and governments have admitted LC-MS/MS with isotope dilution, GC-MS, or GC-MS/MS after derivatization as standard methods for quantification of acrylamide in thermally processed foods (Hu et al. 2015). These methods represent high accuracy and sensitivity as well as good stability and reproducibility that made them reliable for analysis and detection of acrylamide (Pan et al. 2020). However, LC-MS/MS has no derivatization process that decreases the detection time and meets the requirements for a green environment (Fernandes et al. 2019; Jozinović et al. 2019). In addition, due to the good separation effect, LC-MS/MS can be utilized for simultaneous detection of acrylamide and other toxic compounds in one sample (Lee et al. 2015). The European Union (EU) announced that analytical methods used for acrylamide detection should have a limit of quantification (LOQ) of 30 μg/kg for breads and foods for infants. In standard methods such as LC-MS/MS or GC-MS, limit of detection (LOD) and LOQ are in the range of 1–5 and 10–30 μg/kg, respectively, which fulfill the EU recommendations. Moreover, some rapid methods such as electrochemical biosensors, ELISA, fluorescence, and computer vision methods have been found sensitive and selective for acrylamide determination (Oracz et al. 2011).

Acrylamide measurement by LC-MS and GC-MS

Sample preparation, extraction, and clean-up

It is obvious that sample preparation and extraction play an important role in measurement of acrylamide in food matrices (Elbashir et al. 2014). Although depending on the food matrix, there are diverse preparation steps, the common procedures for sample preparation include homogenization of sample, addition of internal standard, defatting, extraction, concentration, and clean-up (Rufián-Henares and Morales 2006; Yusà et al. 2006). For acrylamide quantification, external and internal standard addition is a necessary step. Since external quantitative analysis has poor reproducibility and recovery, an internal standard is usually added to the sample to improve the accuracy and precision of measurement (Zhu et al. 2008). The most commonly used internal standards include isotope-labeled internal standards such as deuterium-labeled ($^2H_3$-

AA or d_3-AA) or carbon-labeled (^{13}C3-AA), and non–isotope-labeled internal standards such as methacrylamide, *N*, *N*-dimethylacrylamide, and acetamide (Kim et al. 2011; Sun et al. 2012).

Extraction of acrylamide can be easily carried out by water as acrylamide is highly soluble and there is no need to adjust pH. However, some organic solvents such as methanol, *n*-propanol, 2-butanone, acetonitrile, and acetone can be utilized in extraction (Keramat et al. 2011b). Mixture of water and acetone (80:20 *v*/*v*) (Faraji et al. 2018), methanol (Eslamizad et al. 2019; Wang et al. 2017), water–methanol (80:20 *v*/*v*) (Norouzi et al. 2018), and water (Boyacı Gündüz and Cengiz 2015; Roszko et al. 2019) have been reported for extraction of acrylamide from different bread types. Afterwards, clarification step is required for the samples rich in protein which is performed via addition of acetonitrile, methanol, ethanol, acetone, sodium chloride, or Carrez reagents followed by centrifugation and/or filtration (Pundir et al. 2019). For foods rich in fat, an elimination step using hexane and cyclohexane is carried out (Wang et al. 2008). In some cases, clean-up procedure consisting of solid-phase extraction (SPE) is also necessary that removes interfering compounds leading to greater precision and accuracy of analysis. Depending on the cartridges used in SPE, this step can be conducted by two possible approaches including (1) absorption of acrylamide from the matrix in the cartridges through hydrogen binding, π–π interface and cation exchange followed by using other polar solvents, and (2) collection of acrylamide in the elution by using cartridges Oasis HLB coupled with Bond Elut-Accucat and a homemade SPE column filled with the mixture of C18, SCX, and SAX (Bortolomeazzi et al. 2012).

It should be taken into account that optimization of extraction conditions (sample grinding, type of solvent, extraction time and temperature, number of extraction cycles and preliminary steps before extraction), clean-up step (type of SPE cartridge), eluent, and filtration has a paramount importance for achieving accurate results (Zhang et al. 2006).

Acrylamide detection and quantification

Among all methods developed for determination of acrylamide, chromatographic methods along with mass technique exhibit good selectivity and sensitivity (Elbashir et al. 2014). It can be elucidated from recent studies that GC-MS and LC-MS coupled with tandem mass spectrometry are the most extensively used method for acrylamide determination (Pundir et al. 2019). In GC-based methods, acrylamide is determined through derivatization with potassium bromate and potassium bromide due to the low volatility and polarity of the molecule. The bromination of acrylamide has advantages including producing less polar molecules compared to the primary acrylamide that can be easily extracted by ethyl acetate or *n*-hexane, eliminating many water-soluble components. Also, this process increases the molecular weight of acrylamide that improves sensitivity and specificity of analysis. In addition, bromination increases the volatility of acrylamide that results in an improved separation during GC analysis (Keramat et al. 2011b; Wenzl et al. 2003; Zhu et al. 2008). However, few studies have reported direct acrylamide analysis without derivatization and using columns with polar phases. Direct analysis of acrylamide via GC-MS has some disadvantages due to the following reasons: (1) absence of characteristic ions in the mass spectrum of underivatized acrylamide and matrix interference that increases background noise and thus achieving low detection limit is impossible (Oracz et al. 2011); (2) potential production of acrylamide in situ in the heated GC injector from co-extracted precursors (asparagine and reducing sugars) when water is used for extraction (Dunovská et al. 2006; Weißhaar 2004); (3) poor retention of underivatized acrylamide on conventional GC columns as a consequence of higher polarity (Castle and Eriksson 2005); (4) co-elution of the compound 3-hydroxypropionitrile with acrylamide, causing overestimation of acrylamide (Zhang et al. 2009).

Lee et al. (2007) used direct immersion solid-phase microextraction (SPME) and gas chromatography–positive chemical ionization tandem mass spectrometry (GC-PCI-MS-MS) for the determination of acrylamide in aqueous matrices. Other detectors that can be coupled with GC are flame-ion detectors or electron capture detectors (ECD), and nitrogen–phosphorus detector (Zhang et al. 2006).

LC-MS/MS method for determination of acrylamide is gaining attention and is currently used in official institution and government departments because of high sensitivity and no need for derivatization. It has been indicated that LC-MS/MS has better between-laboratory performance than GC-MS methods (Wenzl et al. 2006). Current methods based on LC-MS/MS with standard LC columns show good recovery and within-laboratory repeatability with LOD of 3–20 μg/kg and LOQ of 10–50 μg/kg which is proper for the immediate requirements for food product monitoring and calculation of consumer exposure (Eslamizad et al. 2020).

It has been reported that it has high selectivity when used in multiple reaction monitoring (MRM) mode (Keramat et al. 2011b). The most frequent ionization methods in LC-MS include electrospray ionization (ESI) and chemical ionization under atmospheric pressure (APCI), which are considered as mild techniques suitable for polar compounds (Liu et al. 2008). Tables 2 and 3 present selected publications on acrylamide determination in bread samples by LC- and GC-based methods, respectively.

Other methods for quantification of acrylamide

Another technique used in determination of acrylamide is capillary electrophoresis (CE). In this method, compounds with different charges dissolved in the electrolyte and migrate to

Table 2 LC-based analysis for determination of acrylamide in bread

Sample	Sample preparation and clean-up	Internal standard	Column and chromatography parameters	Detector parameters	Reference
Bread rolls	Extraction was performed by addition of 100 mL water to the ground sample in an ultrasonic bath at 40 °C. After Carrez precipitation and filtration, clean-up was carried out by Isolute Multimode cartridges followed by a concentration step using Chem Elute cartridges and ethyl acetate as the eluent	Acrylamide-d3	Hypercarb column (100 × 2.1 mm; 5 mm) (Thermo Hypersil, Dreieich, Germany), equipped with a C18 guard column (4.0 × 3.0 mm) (Phenomenex, Torrance, CA) Mobile phase was 1% acetonitrile/0.05% formic acid (*v*/*v*) in water at a flow rate of 0.2 mL/min, and the total run time was 10 min	MS/MS with electrospray ionization in the positive-ion mode Nitrogen was used both as the drying and nebulizing gas. Helium was used as the collision gas for collision-induced dissociation (CID) at a pressure of 4.0×10^{-6} mbar. Masses were recorded using multiple reaction monitoring (MRM). For quantitation, the signals at *m*/*z* 55.5 (ISTD *m*/*z* 58.5) were used	(Claus et al. 2008b)
Traditional and semi-industrial bread	1 g sample + 100 μL acrylamide-d3 + 2.5 mL methanol followed by centrifugation. Addition of Carrez I and II and shaking Addition of 50 mg primary secondary amine (PSA), shaking and centrifugation at 5000 rpm for 10 min, separation of methanol extract and evaporation by nitrogen gas, and dissolving the remaining extract in water	Acrylamide-d3	ODS-H optimal-C18, Capital (150 mm × 4.6 mm, 3 μm) Isocratic mixture of 0.1% acetic acid in an aqueous solution of formic acid and 3% methanol (97:3, *v*/*v*) at a flow rate of 0.5 mL/min	MS/MS with electrospray ionization in the positive-ion mode Capillary set at 4.0 kV and the collision energy at 10 eV. The source gas temperature was set at 325 °C and the desolvation temperature was set at 400 °C. Nitrogen was used as a nebulizer gas (flow 10 L/min) and desolvation gas (flow 150 L/h) The multiple reaction monitoring (MRM) mode of the degradation patterns *m*/*z* 72 → 55 (acrylamide) and *m*/*z* 75 → 58 (acrylamide-d3) were used for quantification	(Eslamizad et al. 2019)
Leavened and unleavened bread	1 g sample + 5 mL methanol and centrifugation (10,000×*g* for 10 min) after 2 min. Addition of Carrez I and II to the supernatant and centrifugation at 5000×*g* for 10 min. Collection of supernatant and evaporation to dryness under a gentle stream of nitrogen at 40 °C and dissolving the residue in 1 mL of distilled water Clean-up performed by SPE.	–	Hypersil ODS-C18 column (250 mm × 4.6 mm, 5 mm; Thermo Scientific, Waltham, MA, USA) at 40 °C The mobile phase was a mixture of acetonitrile and water (5:95, *v*/*v*), and the flow rate was 0.6 mL/min	UV wavelength of 210 nm	(Wang et al. 2017)
Bread	Addition of acetonitrile and QuEChERS salt mixture (4 g anhydrous $MgSO_4$ and 0.5 g of NaCl to sample. Purification of		Luna 3 μm HILIC column (100 × 3.00 mm) (Phenomenex, Torrance, CA, USA)		(Nachi et al. 2018)

Table 2 (continued)

Sample	Sample preparation and clean-up	Internal standard	Column and chromatography parameters	Detector parameters	Reference
	the extract was conducted by 50 mg of PCA-sorbent and 150 mg anhydrous $MgSO_4$		The solvent system was composed of 0.1% formic acid in water and 0.1% formic acid in methanol with a flow rate of 0.3 mL/min		
Baked bun	3 g sample + 30 mL water and internal standard, homogenized and centrifuged at 3424×*g* for 10 min and the supernatant used for analysis SPE clean-up was performed using Isolute M-M 300 mg 3 mL SPE columns	*d3*-acrylamide	0.1% aqueous formic acid at 0.2 mL/min on a Hypercarb column (dimensions 2.1 × 100 mm, particle size 5 μm)	MS/MS with electrospray ionization in the positive-ion mode ESI+ using *m*/*z* 72 → 55 of acrylamide relative to *m*/*z* 75 → 58 of *d3*-acrylamide for quantification and capillary voltage of 3.00 kV	(Katsaiti and Granby 2016)
Bread crisps	0.45 g sample + 5 mL water + internal standard and homogenized. Carrez solutions were added and centrifuged at 4000 rpm for 10 min Clean-up was performed using SPE by Isolute HLB cartridge	[2,3,3-d3]-acrylamide	Inertsil column (25 × 0.46 cm, 5 μm) using mobile phases of 0.2% formic acid in water (solvent A) and methanol (solvent B) The gradient elution applied was 0–3 min 100% A, 3–8 min 93% A, and 8–12 min 100% A, at a flow rate of 0.8 mL/min	MS/MS with electrospray ionization in the positive-ion mode Quantification was conducted in MRM mode at *m*/*z* ratios of 72.1 and 75.1 for acrylamide and [2,3,3-d3]-acrylamide, respectively Monitoring was done at *m*/*z* 55.1 and 44.0 and at *m*/*z* of 58.0 and 44.0 corresponding to specific molecular fragments of acrylamide and [2,3,3-d3]-acrylamide, respectively	(Capuano et al. 2010)
Wheat bread, bran bread, whole wheat bread	6 g sample + 30 mL water and shaken for 30 min. Carrez solutions were added and centrifuged at 10,400 g for 15 min. Defatting was performed by addition of hexane to the supernatant. 2 mL internal standard was added to 20 mL of aqueous phase followed by addition of HCl and dilution with water SPE clean-up was carried out by OASIS MCX cartridge.	[2,3,3-d3]-acrylamide	Zorbax column C18 (50 mm × 4.6 mm, 1.8 μm; Santa Clara, USA) using the isocratic mixture at a flow rate of 0.8 mL min^{-1} Solvent A was 0.3% (*v*/*v*) aqueous formic acid solution and solvent B was acetonitrile in a ratio of 90:10 (*v*/*v*)	MS/MS detector in MRM mode at transition ions of *m*/*z* 72.0 → 55.0 for acrylamide and *m*/*z* 75.1 → 58.0 for D3-AA Drying gas temperature (N2) of 350 °C with a flow rate of 12 L min^{-1}, nebulizer pressure of 275.8 kPa, capillary voltage of 4 kV, fragmentor voltage of 60 V for D3-AA and 70 V for acrylamide, collision energy of 10 V for each transition	(Alpözen et al. 2015)
Portuguese bread	2 g sample + 20 mL water/formic acid (0.1%) and shaken for 2 min and then in an oscillating shaker for 30 min at 70 oscillations per minute followed by	–	UPLC BEH C18 column (2.1 × 50 mm) Isocratic elution with 90% water and 10% acetonitrile at a flow rate of 0.2 mL/min	MS/MS with electrospray ionization in the positive-ion mode Capillary voltage of 3 kV, cone voltage 29 V, source temperature 120 °C, desolvation gas temperature 350 °C,	(Jesus et al. 2018)

Table 2 (continued)

Sample	Sample preparation and clean-up	Internal standard	Column and chromatography parameters	Detector parameters	Reference
	centrifugation at 10,000 rpm for 15 min SPE clean-up was carried out by Oasis HLB SPE cartridge			desolvation gas flow of 5 L/h, cone gas flow at 30 L/h and the pressure of the collision gas was 3×10^{-3} mbar	
Wheat bread	1 mL internal standard and 9 mL water was added to 1 g sample, homogenized for 5 min and centrifuged at 10,000×*g* for 20 min at 5 °C and filtered SPE clean-up was carried out by Oasis HLB SPE cartridge	^{13}C3-acrylamide	Waters Atlantis dC18 column (2.1 × 150 mm), the mobile phase was 10% methanol with 0.1% formic acid, and the flow rate was 0.2 mL/min	MS/MS with electrospray ionization in the positive-ion mode Capillary voltage of 2.5 kV, cone voltage of 20 V, source temperature of 120 °C, desolvation gas temperature of 250 °C, and flow rates of cone and desolvation gas (nitrogen) of 100 and 800 L/h, respectively; MRM was acquired with the characteristic fragmentation transitions *m*/*z* 72 → 55 for acrylamide and *m*/*z* 75 → 58 for ^{13}C3-acrylamide	(Mildner-Szkudlarz et al. 2019)
Wheat bread Rye bread	2 g of sample + internal standard (100 ng/g) + 5 mL of hexane were vortexed. 10 mL distilled water and 10 mL acetonitrile were added, followed by the QuEChERS extraction salt mixture (4.0 g of anhydrous $MgSO_4$ and 0.5 g of NaCl). The sample tube was shaken and centrifuged at 4500×*g* for 5 min. The hexane layer was discarded and 1 mL of the acetonitrile extract was transferred to a tube containing 50 mg of PSA sorbent and 150 mg of anhydrous $MgSO_4$	(^{13}C3-acrylamide	Luna 3 μm HILIC dC18 column (50 × 2.00 mm i.d., 3 mm; Phenomenex, Macclesfield, UK) Methanol (6%) in acidified acetonitrile (0.1% acetic acid) was used as a mobile phase (flow rate 0.25 mL/min, column temperature 40 °C)	MS/MS were as follows: ionization was performed using electrospray in the positive mode (source temperature 120 °C, desolvation temperature 400 °C; cone gas flow 25 L/h, desolvation gas flow 600 L/h)	(Bartkiene et al. 2013)
White wheat, wheat bran, and whole-grain wheat breads	20 mL methanol was added to 3 g sample and vortexed for 20 min then centrifuged at 9000 rpm for 10 min. Carrez solutions were added to the supernatant and centrifuged again The supernatant was evaporated by nitrogen and the residue dissolved in water SPE clean-up was carried out by Oasis HLB SPE cartridge	–	Zorbax SB-C18 column (250 mm, 4.6 mm i.d. and 5 μm particle size) (Agilent). Mobile phase was consisting of 0.2% formic acid and 2% acetonitrile in water. It was delivered isocratically at 0.5 mL/min flow rate. The column temperature was maintained at 40 °C	MS/MS with electrospray ionization in the positive-ion mode Nebulizer gas pressure, dry gas flow rate, and dry gas temperature were set at 45 psi, 10 L/min, and 350 °C, respectively. Detection and quantification of acrylamide in samples were performed with the protonated molecular ion signals at *m*/*z* 72	(Tuncel et al. 2010)

Table 3 GC-based analysis for determination of acrylamide in bread

Sample	Sample preparation	Internal standard	Derivatization	Chromatography parameters/detector	Reference
Iranian bread samples	1 g sample + 4 mL water–methanol (80:20) + spiking internal standard. Immersion of sample into an ultrasonic water bath for 5 min at 40 kHz of ultrasound frequency and 0.138 kW of power at 25 °C. Centrifugation of the solution for 5 min at 2683.2×*g* and separation of upper phase Addition of Carrez I and II for precipitation of proteins and carbohydrates This sample was shaken for 1 min, centrifuged for 5 min at 2683.2×*g*, and upper solution was separated	Acetamide	60 μL of xanthydrol, 5% in methanol, and 2 mL of hydrochloric acid (1 mol L^{-1}) at room temperature and darkness for 30 min	MS/MS HP-5MS capillary column with 5% phenyl siloxane/95% methyl polyorganosiloxane, internal diameter 30 m × 0.25 mm and film thickness 0.25 μm 100 °C held for 1 min, ramped to 300 °C at 20 °C/min, and held for 10 min Injector temperature and the auxiliary temperature were set at 290 and 280 °C, respectively	(Norouzi et al. 2018)
Commonly consumed bread types in Turkey	1 g ground sample suspended in 8.2 mL water at 60 °C followed by addition of internal standard and Carrez solutions and centrifugation at 8000 rpm for 30 min. 3 mL of clarified aqueous layer filtered through a 0.45-μm syringe filter	$^{13}C_3$-acrylamide	300 μL of bromine solution for 1 h in the dark to 2-BPA	MS/MS Capillary column (TR-WAX, 30 m × 0.25 mm × 0.25 μm) A 50 °C initial temperature was held for 1 min, then increased to 180 °C at 20 °C/min, then to 260 °C at a rate of 10 °C/min, and held for 10 min at this temperature	(Boyacı Gündüz and Cengiz 2015)
Wheat soft bread	2.5 g sample spiked with IS + 40 mL water, sonicated for 30 min + Carrez solutions, centrifugation at 1000×*g* Clean-up was performed by SPE	–	2 g of potassium bromide, 0.75 mL of concentrated HBr, and 2 mL of bromine water were kept in the dark for at least 2 h	MS/MS HP Innowax (30 m × 0.25 mm × 0.25 μm; Agilent, Santa Clara, CA, USA) polyethylene glycol-based fused-silica capillary column 1 min hold at 40 °C, 15 °C/min ramp to 120 °C (2 min hold), 7 °C/min ramp to 160 °C, 3 °C/min ramp to 180 °C, 20 °C/min ramp to 230 °C (5 min hold)	(Roszko et al. 2019)
Bread	Weighed sample + 30 mL water + internal standard at 60 °C. 20–40 μL acetic acid was added followed by Carrez solutions and centrifuged at 6000×*g* for 30 min	1,2,3-^{13}C labeled acrylamide	Addition of 7.5 g KBr, 40–100 μL HBr (pH 1–3), 10 mL saturated bromine-water solution on a shaking water bath below 4 °C, for at least 2 h and formation of 2,3--dibromopropionamide (2,3-DBPA)	MS/MS Positive ionization mode (EI+); acquisition mode: "selected reaction monitoring-SRM" Capillary column based on polyethylene-glycol (30 m × 0.25 mm internal diameter; 0.25 μm) (TraceGOLD TG-WaxMS; Thermo Fisher Scientific, USA).	(Negoiță and Culețu 2016)
Whole wheat bread	10 g sample + 10 μg/mL acrylamide solution + distilled water and mixing for 30 min and filtered 25 mL hexane was added to 25 mL of the filtrate and mixed for 2 min and the aqueous phase separated	–	Addition of 7.5 g KBr, 0.4 μL HBr (pH 1–3), 8 mL saturated bromine water and stirred for 1 h in ice bath leading to formation of 2,3-DBPA and finally 2-BPA	Electron capture detector (ECD) DB-WAX capillary column (polyethylene glycol, 30 m × 0.25 mm) Internal diameter [i.d.] column was held at 60 °C for 1 min, then programmed at 20 °C/min to 220 °C and held for 3 min at 220 °C, then at 30 °C/min to 250 °C and held for 5 min at 250 °C	(Nasiri Esfahani et al. 2017)

electrodes at different rates by applying a high voltage (Kataoka et al. 2009; Zhou et al. 2007). Capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MEKCC) are two variants of CE. Bermudo et al. (2006) utilized CZE and derivatization with 2-mercaptobenzoic acid to specify acrylamide in food products including biscuits, crisp bread, cereal flakes, potato crisps, and coffee. In another study by Kruchina-Bogdanov et al. (2018), CE with spectrophotometric detection was used to determine acrylamide level in bakery products made of wheat flour and sea-buckthorn powder.

Furthermore, due to some drawbacks in chromatographic methods such as a long time required for sample preparation, expensive equipment, and high analysis costs, some rapid methods including color indicating methods, ELISA, supramolecular recognition-based methods, and various biosensors have been developed (Hu et al. 2015; Pundir et al. 2019).

According to Hu et al. (2015), rapid methods such as electrochemical biosensors have LOD value about two orders of magnitude lower than standard methods (LC-MS- and GC-MS-based methods) (Hu et al. 2015). Based on data for recoveries (inter- and intra-) and relative standard deviations (RSD), standard methods are more stable, repeatable, and reproducible than rapid methods. The RSD values of standard methods are lower than 10% or even 5%. However, the lack of data related to inter- and intra-tests for rapid methods demonstrates that the repeatability needs further investigations. Regarding the type of food product, diverse products such as potato chips, cereal-based foods, coffee, tea, and noodles have been examined by standard methods, but in the case of rapid methods, potato chips have been used for evaluating their application and this topic should be improved in these methods. In standard methods, purification of SPE cartridges is essential for high selectivity that raises the cost and complexity of processes, but rapid methods have simpler pretreatment based on biochemical characteristics that decrease the cost of detection. Regarding the time required for acrylamide detection, the pretreatment of samples is the main time-consuming step in standard methods. In contrast, in rapid methods, a simple or no pretreatment is needed that decreases detection time by 40% or more. Moreover, standard methods require expensive instruments and skilled labor that limit their application to laboratory, but simple procedures and portable instruments along with nanotechnology enable rapid methods the possibility to achieve on-line and real-time detection. Nevertheless, rapid methods still need to be optimized due to their ambiguous repeatability and precision, and their analytical results should be confirmed by other robust methods.

Conclusion

Acrylamide as a carcinogen, mutagen, and reproductive toxicant is produced in low-moisture-content food products exposed to high temperature. The high protein and carbohydrate content of bread and its baking process make it susceptible for formation of acrylamide. This compound does not have any threshold in its dose–response relationship which shows that even one molecule of it could cause a problem via interaction between its active metabolite (GA) and vital molecules in the body such as DNA. The risk of carcinogenicity through acrylamide via bread consumption is potential while the risk of neurotoxicity was negligible in most previous studies. Despite low acrylamide content of bread, it has been considered as the main acrylamide exposure source regarding its fairly extreme consumption rate especially in developing countries that are on the basis of cereal-based diet. Determination of acrylamide content is critically important for both the risk assessment and quality control aspects. Several analytical methods have been investigated for acrylamide determination including LC-MS, GC-MS, and capillary electrophoresis. Reduction of acrylamide level in breads seems to be possible by changing the raw materials, optimizing the processing conditions, and using additives as well as fermentation.

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