New Research Developments on Acrylamide: Analytical Chemistry, Formation Mechanism, and Mitigation Recipes

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Received April 19, 2008

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1. Introduction

Acrylamide, a colorless and odorless crystalline powder with a melting point of 84.5 °C, is soluble in water, acetone, and ethanol. Acrylamide has a high mobility in soil and groundwater and is biodegradable.[1] Long-term exposure to acrylamide may cause damage to the nervous system both in humans and in animals to a certain extent.[2] Meanwhile, acrylamide is also regarded as a potentially genetic and reproductive toxin[3] with mutagenic and carcinogenic properties in both *in vitro* and *in vivo* studies.[4] The risk of acrylamide estimated by the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) in the European Union (EU) demonstrated that the exposure of acrylamide to humans should be controlled as low as possible with regard to its inherently toxic properties, including neurotoxicity, genotoxicity to both somatic and germ cells, carcinogenicity, and reproductive toxicity.[5]

In 2002, acrylamide was widely found in carbohydrate-rich foods and evoked an international health alarm.[6] It was

10.1021/cr800318s CCC: $71.50 © 2009 American Chemical Society
Published on Web 08/07/2009

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well-known that acrylamide is mainly generated from the Maillard reaction between asparagine and carbohydrates.[7] Some book chapters and special issues summarized the state-of-the-art about all aspects of dietary acrylamide. A monograph named "Chemistry and safety of acrylamide in food" was published,[8] which systematically introduced the discovery, analytical method, occurrence, toxicology, metabolism, and risk assessment of acrylamide in heat processing foods. Moreover, a special edition of *The Journal of AOAC International* was devoted to a summarization on 2-year

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research activities.[9] In this issue, the risk management,[10] exposure mitigation,[11] stability during storage,[12] perspective in formation mechanism,[13] analysis,[14] monitoring databases,[15] activities conducted by the food industry,[16] and evaluation of results coming from proficiency tests[17] of acrylamide were discussed. With the research development of acrylamide contaminant in various foods, many review articles focused on some special fields and gave an overall picture of research progress to the audience (Table 1). Recently, many important developments such as rapid analytical methods, new formation pathways, and mitigation recipes via additives were highlighted, which contributed to the advances in acrylamide research. These latest findings deserve to be reviewed and summarized.

This review will address new analysis, formation, and mitigation aspects of the acrylamide issue and summarize the progress made to date in these key areas on a chemical basis. These contents were not reviewed in previously published review articles. The main cited references were published during 2006−2008. The new analytical methods include capillary zone electrophoresis, liquid chromatography with pulsed electrochemical detection, time-of-flight mass spectrometry, etc. The new formation studies include some other formation pathways such as the acrolein pathway, the triglyceride pathway, and related in-depth mechanisms other than the Maillard reaction pathway. The new mitigation studies include the application of additives such as antioxidants, polyphenols, and salts and their mitigation mechanism such as the antioxidant paradox and the quinone−amine interaction. Meanwhile, some kinetic models recently established will be introduced, such as the mechanistic kinetic model and the logic-Fermi model.

2. Analytical Chemistry of Acrylamide

Before the finding of acrylamide in heat processing foods in 2002, some respective methods based on LC or GC techniques were established to determine the acrylamide contents in several products, such as mushrooms,[38] field crops,[39] and sugars.[40] However, these methods cannot be competent for the analysis of acrylamide in heat processing foods at trace levels. The MS-based chromatographic methods were then developed for the analysis of acrylamide. It

Table 1. Summarizations of Published Review Articles for All Aspects of Acrylamide

authors	main review contents	ref
Besaratinia and Pfeifer	DNA adduction and mutagenic properties	18
Blank	state-of-the-art review on analysis, safe assessment, and formation	19
Claeys et al.	quantification, formation, and kinetics during food processing	20
Claus et al.	toxicology, formation, and reduction methods in cereal products	21
Corradini and Peleg	linear and nonlinear kinetics in the synthesis and degradation	22
Dybing et al.	human exposure and internal dose assessments in food	23
Friedman	chemistry, analysis, formation mechanism, biochemistry, and safety	24
Friedman and Levin	reduction and toxicity	25
Guenther et al.	analysis, formation, and reduction level in coffee	26
Klaunig	carcinogenicity	27
Konings et al.	reduction level in cereal and cereal products	28
LoPachin et al.	neurotoxicology hypothesis	29
Parzefall	the toxicity and dietary exposure	30
Rice	carcinogenicity	31
Shipp et al.	toxicity and dose−response analysis for cancer and noncancer effects	32
Taeymans et al.	analysis, formation, and control with an industry perspective	33
Stadler and Scholz	analysis, levels in food, formation mechanism, and control strategies	34
Wenzl et al.	analytical methods	35
Zhang et al.	analytical methods	36
Zhang et al.	formation mechanism, impact factors of formation, and mitigation	37

can be summarized from recent methodological studies that GC-MS and LC-MS/MS appeared to be acknowledged as the most useful and authoritative methods for the quantification of acrylamide.[35,36] In 2005, WHO and FAO together announced that certain foods, especially Western-style snacks processed or cooked at high temperature, contain large amounts of acrylamide and may harm human health to a certain extent.[41] Therefore, research on acrylamide in different food matrices has once again attracted extensive attention. For the analytical methods, the improvement of MS-based techniques and validation of non-MS based quantitative methods were greatly concerned. Besides the repeatability, sensitivity, and precision, the convenience and rapid analysis of methods were also taken into consideration.

2.1. General Knowledge of Pretreatment and Analytical Methods

2.1.1. General Pretreatment Steps

All the extraction and cleanup steps of acrylamide generally summarized from many peer-reviewed papers before sample injection of GC-MS or LC-MS/MS analysis are shown in Figure 1. Water at room temperature has been used to extract acrylamide from various sample matrices in most published analytical methods because acrylamide is a good hydrophilic small molecule.[42] Besides, methanol also can be used to extract acrylamide for the convenience of rotatory evaporation and concentration.[43] Young et al.[44] suggested that acrylamide could be extracted by a high level aqueous solution of sodium chloride so that the emulsification process was obviously reduced and the high recovery of acrylamide was observed. Moreover, one of the laboratories joined the proficiency test of acrylamide and used a mixture of water and acetone as extractant.[45] For the development of the extract method of acrylamide, some publications[46] mentioned accelerated solvent extraction (ASE). ASE is a technique for extracting solid and semisolid samples with liquid solvents. ASE uses conventional liquid solvents at elevated temperatures and pressures to increase the efficiency of the extraction process. Increased temperature accelerates the extraction kinetics, while elevated pressure keeps the solvent below its boiling point, thus enabling safe and rapid extractions.

In order to control the recoveries and keep track of possible losses occurring during the whole sample pretreatment, an internal standard was added to the sample matrix after homogenization. Most published studies used $^{13}C_3$-acrylamide produced by Cambridge Isotope Laboratories (Andover, MA) as internal standard.[42a,47] Besides this, $^{13}C_1$-acrylamide,[48] D_3-acrylamide,[49] *N*,*N*-dimethylacrylamide,[42c,47e] methacrylamide,[50] and propionamide[51] also have been published to be used as acrylamide internal standard.

Some researches about acrylamide analysis include a defatting step before or in combination with the extraction step. This is carried out by extraction with hexane, petroleum ether, or cyclohexane. Moreover, some protein-rich sample matrices need a deproteinating step. This is carried out by adding a high level of methanol, acetonitrile, or saline solution. Delatour et al.[52] achieved the visual protein precipitation step within <1 min by the addition of potassium hexacyanoferrate(II) trihydrate (Carrez I) and zinc sulfate heptahydrate (Carrez II) under continuous swirling. The similar protein precipitation step was achieved by Gertz and Klostermann.[53] Whether the extraction step of acrylamide needs a defatting or deproteinating step should be carried out according to sample matrices.

Most cleanup procedures consist of the combination of several solid-phase extractions (SPE). Becalski et al.[42a] used a combination of three different cartridges: Oasis MAX (mixed-mode anion exchange), Oasis MCX (mixed-mode cation exchange), and ENVI-Carb (graphitized carbon). A similar combination of SPE cartridges consisting of Bond Elut C_{18}, Bond Elut Jr-PSA (anion exchange), and Bond Elut Accucat was chosen for cleanup of samples, which were measured by LC-MS with column switching.[48b] An oasis HLB cartridge, the main supporter of which is a hydrophilic−lipophilic balance and water-wettable reversed-phase sorbent for all compounds and all of general SPE needs, is in common use during the SPE cleanup step of acrylamide analysis. Many researchers reported the application of this cartridge before the chromatographic analysis of acrylamide.[54] An oasis MCX cartridge, the main supporter of which is a mixed-mode cation-exchange reversed-phase sorbent for bases with a high selectivity for basic compounds, also can be used. Good accuracy and high recovery of chromatographic method

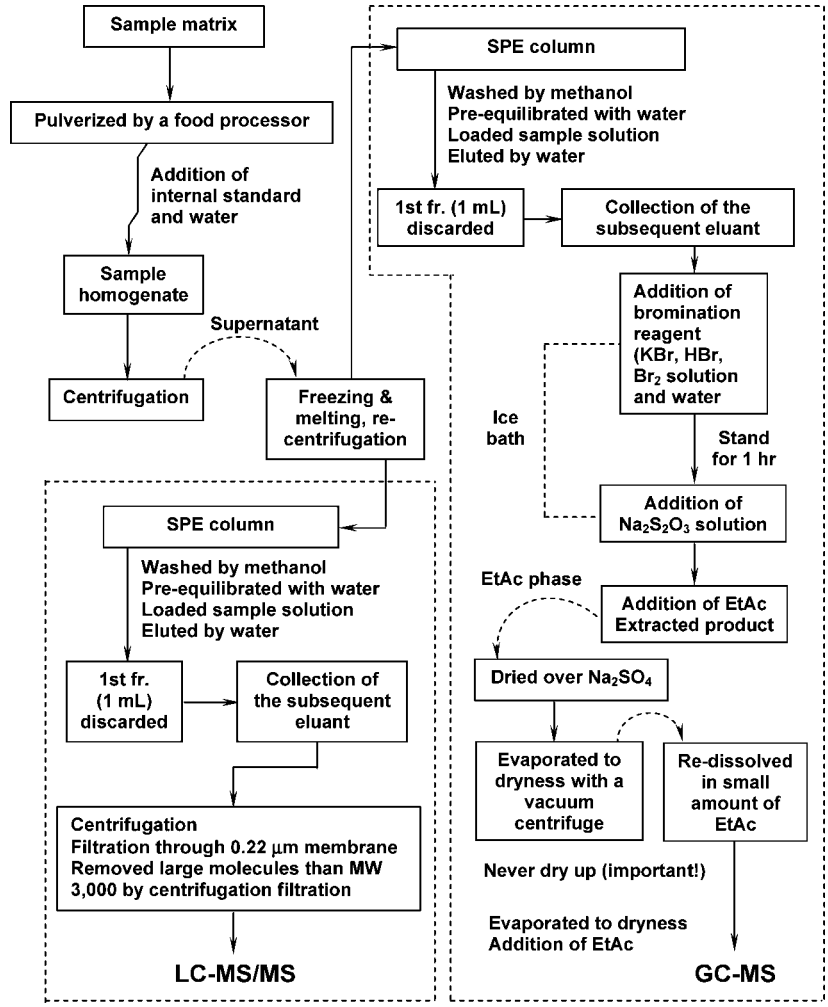

Figure 1. Representative steps of sample pretreatment before the determination of acrylamide.

validation during the acrylamide analysis can be achieved when reasonable and effective cleanup procedures are well designed.

2.1.2. General Analytical Methods

An isotope dilution liquid chromatography mass spectrometry (LC-MS) method for the quantitative analysis of acrylamide in various heat processing foods was first reported by Rosén and Hellenäs.[42b] From then on, a sequence of analytical methods dealing with the determination of acrylamide in heat processing foods have been published in peer reviewed journals, reported by specific research groups, or presented at international scientific conferences.[35] Most of the reported analytical methods are based on MS as the determinative technique, coupled with a chromatographic step either by LC[42b,47d,55] or GC with[47e,49a,53,56] and without[43a,57] derivatization.

2.1.3. FAPAS Proficiency Test

The need for a certified matrix reference material of acrylamide in a food matrix is emphasized by the competent authorities as a tool to improve comparability, ensuring accuracy and traceability of analytical results. The production of such a reference material is mainly according to ISO Guide 34 (2000), *General requirements for the competence of reference materials producers*, and ISO Guide 35 (2006), *General and statistical principles for certification*, which include the material processing, homogeneity, stability assessment, material characterization, and the acrylamide mass fraction value assignment.[58] The validation can be performed when the analysis is integrated within the scope of an authorized proficiency test controlled by the official Food Analysis Performance Assessment Scheme (FAPAS) for accreditation.[45] The decision of such a test will be made by FAPAS after comparing the quantitative results with the measurement results from individual laboratories and the assignment value. Although such a proficiency test is not a prerequisite for performing the quantification of acrylamide, the analytical accuracy and repeatability will be compellent if a high score of such a test for the determination of a reference material is approved by FAPAS.

2.2. New Developments on Pretreatment Steps

2.2.1. Optimization of Extraction Procedure

The extraction procedure is an important step at the beginning of sample pretreatments. Besides the choice of extraction solvents, other extraction factors including sample particle size, defatting, use of Ultra Turrax during homogenization, extraction temperature, and extraction time should also be considered. Generally, incomplete extraction is the most likely cause of erroneous results for the analysis of acrylamide. This might occur when the food is not sufficiently macerated and when using a short extraction time or low extraction temperature, especially when these condi-

tions are combined.[59] Formation of acrylamide during the extraction procedure may be another possible error source, which is an easily neglected factor.[60] Furthermore, the method for extraction of dietary fibers or at high pH can affect the analytical results.[61] Other possible pitfalls during the extraction procedure include contamination of acrylamide from labware such as syringe filters and ultrafilters[62] and thermal degradation of acrylamide. Recently, some studies found that acrylamide can significantly coevaporate with water.[63] Therefore, too high an extraction temperature should be avoided. To improve the extraction efficiency, a pressurized fluid extraction step with acetonitrile can be used.[64]

2.2.2. Optimization of Solid-Phase Extraction

During these years, the use of multiple cartridges during SPE is still widespread because the advantage of different cartridges is combined to obtain a high recovery of acrylamide, such as the combination of ENV+ (a cross-linked polystyrene-based polymer) and Strata-X-C (a cation exchange polymer).[65] As an improved technique, layered SPE cartridges are used to reduce the cleanup procedures. Soares et al.[66] found that adding a layer of C_{18} sorbent to the Isolute Multimode sorbent with a ratio of 1:3 is ideal to eliminate the most relevant contaminants in some complex food matrices, such as coffee. To consider the balance between the experimental cost and reasonable recovery, single SPE cartridges are also used. Under such a situation, Isolute-Multimode (a hydrophobic interaction sorbent)[67] and oasis HLB[68] cartridges are widely applied. Besides routine SPE, some other techniques including solid-phase microextraction (SPME)[69] and matrix solid-phase dispersion (MSPD)[70] were also optimized and successfully applied during sample preparation. To improve the reliability of analysis methods applied to food samples, Kim et al.[71] developed an LC-MS/MS method and determined the recovery efficiency using d_5-3-chloropropanediol as a recovery standard during SPE. The ion transitions of m/z 116 > 98 (d_5-3-chloropropanediol) were found to be the most reliable for the identification of acrylamide in multiple reaction monitoring (MRM). The use of d_5-3-chloropropanediol minimizes the effects of variation in the sample matrices and increases the quality of analysis.

2.2.3. Special Attraction for the Analysis of Biological Samples

With respect to the risk assessment of the general population, the estimization of the internal exposure is of great importance. For the analysis of biological samples, the mercapturic acids and hemoglobin adducts of acrylamide and glycidamide in urine and blood samples are especially attractive. The most prominent metabolites excreted in urine are the mercapturic acids of acrylamide, *N*-acetyl-*S*-(2-carbamoylethyl)-L-cysteine (AAMA), and of glycidamide, *N*-(*R/S*)-acetyl-*S*-(2-carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA), according to animal studies.[72] For the pretreatment of urine samples, formic acid and ammonium formate buffer (pH ~2.5),[73] or hydrochloric acid[74] can be used for the precipitation of protein. Besides, addition of deuterium-labeled internal standard and SPE cleanup are necessary for the analytical accuracy and sufficient removal of coextractives. The pretreatment of blood samples is relatively complex, including blood treatment, isolation of globin, and modified Edman degradation.[75]

2.3. New Developments on Instrumental Analysis

2.3.1. Improvement of GC-MS Methods

GC-based methods usually demand derivatization of acrylamide, which is well performed with hydrobromic acid and saturated Br_2 solution in many laboratories.[35a,42a,c,47c,49a,50] The excess bromine is then removed by addition of sodium thiosulfate until the solution becomes colorless so that the derivative reaction is terminated. The GC-MS methods with or without derivatization of acrylamide were systematically reviewed by Castle and Eriksson.[14] Recently, such a derivatization method was improved and achieved via the reaction between potassium bromate ($KBrO_3$) and potassium bromide (KBr) in an acidic medium.[68d,76] The use of the strong acid (HBr) and saturated Br_2 solution can be avoided whereas these two solvents have been prepared with difficulty, and they are hazardous to handle. Nevertheless, use of the $KBrO_3$ and KBr combination is relatively more convenient and safe, and the reaction is performed in about 30 min at cold storage temperature with excellent reproducibility. For the chromatographic conditions, polar columns of the Carbowax type are mainly applied to chromatographic separation and chemical ionization mass spectrometry in selected ion monitoring mode for analyte detection.[35b,77] During GC-MS analysis, the coelution of some impurities may interfere with the quantitative results of acrylamide. Biedermann and Grob[77] found that 3-hydroxypropionitrile (3-HPN) may be coeluted with acrylamide, causing falsely high acrylamide values. To eliminate such a problem, they improved the method and selected the Carbowax 1000 column with higher polarity. Results showed that acrylamide is eluted clearly after 3-HPN. Alternatively, it is also possible to get 3-HPN eluted after acrylamide using a high molecular weight Carbowax combined with adequate tuning of the separation conditions. Overall, the current robust GC-MS method with optimized derivatizatrion and sample pretreatment procedures could greatly improve the selectivity, sensitivity, and repeatability. The method could also be applied to the quantification of acrylamide in some new matrices, such as malt[78] and cigarette mainstream smoke.[79]

2.3.2. Improvement of LC-MS/MS Methods

The LC-MS/MS methods are in principle based on the method published by Rosén and Hellenäs,[42b] and they are largely improved by various reports.[35,36] For the chromatographic improvements, the retention of acrylamide in the column greatly affects the separation efficiency and quantitative precision. Rosén et al.[80] comparatively investigated the effects of the solid phase on the chromatographic retention of acrylamide. During SPE, a hydroxylated polystyrene−divinylbenzene copolymer phase (ENV+) gives the strongest retention. During HPLC, the best retention is achieved with a phase comprising porous graphitic carbon (Hypercarb) using water as the mobile phase. For the mass spectrometry improvements, the effect of different atmosphere−pressure interfaces on the LC-MS/MS determination of acrylamide was further demonstrated. Marín et al.[81] recommended the Ion Sabre atmospheric-pressure chemical ionization (APCI) as the interface and obtained the highest sensitivity for acrylamide (LOD 0.03 μg/L) and the absence of matrix effects compared to electrospray ionization (ESI) and general APCI. In different foods and model systems, the formation of acrylamide has been shown to correlate with preprocessing levels of asparagine, fructose, glucose, or the product of

asparagine and reducing sugars. Therefore, the change of asparagine and sugar contents is greatly related to the acrylamide level. Previous studies used HPLC and an amino acid analysis kit to quantify the contents of sugars and asparagine, respectively.[82] However, such a method costs considerable time and needs the analysis of large amounts of samples. Nielsen et al.[83] developed a LC-MS/MS method for simultaneous analysis of acrylamide (LOD 0.013 mg/kg), asparagine (LOD 1.8 mg/kg), glucose (LOD 96 mg/kg), fructose (LOD 552 mg/kg), and sucrose (LOD 23 mg/kg) in bread. This method is useful for further investigation of acrylamide formation in various food matrices. With the development of a chromatographic technique, many interference-free, generic, and robust LC-MS[84] or LC-MS/MS[85] methods are validated, which can be regarded as candidate reference methods for the quantification of acrylamide.

2.3.3. New Analytical Methods

Several MS based methods have been developed in the past decades to determine acrylamide in water, foods, and biological samples. Besides, some new analytical methods have recently been validated, including capillary zone electrophoresis (CZE), micellar eletrokinetic capillary chromatography (MEKCC), time-of-flight mass spectrometry (TOF-MS), near-infrared spectrometry, computer vision-based image analysis, etc.

Regarding the capillary electrophoresis (CE) techniques, microemulsion eletrokinetic chromatography (MEEKC) was first proposed for the determination of acrylamide without derivatization,[86] but this method provides a poor LOD value. An improved MEKCC method was developed for the separation and quantification of acrylamide and approved as a reliable method (LOD 0.1 μg/mL).[87] Based on the above techniques, better results were obtained for CZE, after derivatization with 2-mercaptobenzoic acid to obtain an ionic compound (LOD 0.07 μg/mL).[88] To further improve detection limits and to spread the applicability of the method over a wide range of samples, field amplified sample injection (FASI) was proposed (LOD 1 μg/L).[89] Based on the FASI-CE technique, Bermudo et al.[90] showed the applicability of CE coupled to MS/MS for the analysis of acrylamide in foodstuffs and obtained good linearity and precision. Besides the FASI-CE method, a nonaqueous CE method[91] and relative field amplified sample stacking (FASS) technique[92] were also developed and regarded as a simple, rapid, and inexpensive choice.

Considering rapid development of the TOF-MS technique, the LC or GC method combined with high resolution TOF-MS[93] was used for the analysis of acrylamide and validated by FAPAS. Compared to robust GC-MS or LC-MS/MS, the applicability of TOF-MS for the quantification of acrylamide still needs to be optimized and improved in the near future. Another area of research progress is the estimation of acrylamide using the mathematical model and algorithm. Using near-infrared spectroscopy[94] and computer vision-based image analysis,[95] the acrylamide content was modeled and estimated by multiple linear regression and a semiautomatic segmentation algorithm, respectively. Compared to the experimental determination method, such a model method is more fast and convenient, especially when applied to the preliminary estimation and screening of acrylamide content. However, the precision of such an estimation needs to be carefully validated.

2.4. Rapid and Easy-to-Use Methods

For the sample pretreatment, the routine procedure prior to instrumental analysis includes sample preparation, addition of internal standard (IS), defatting, extraction, concentration, and cleanup. Such a tedious procedure is not suitable for the analysis of large amounts of samples. Mastovska and Lehotay[96] optimized a fast and easy procedure that deuterated acrylamide IS was added to homogenized sample together with hexane, water, acetonitrile, magnesium sulfate, and sodium chloride, followed by a dispersive SPE step. Such a procedure avoids time- and labor-intensive steps such as evaporation/solvent exchange, filtration, quantitative transfers, and multiple SPE cleanups using traditional cartridges. For the chromatographic analysis, an improved method, ultraperformance liquid chromatography (UPLC) coupled to MS/MS, was developed.[49c] Compared to routine LC-MS/MS, the UPLC-MS/MS method supplies a rapid quantitative procedure of acrylamide with a run time of only 3 min. Furthermore, it was approved from the chromatogram that the hybrid particles used in UPLC columns often show unique selectivity compared to conventional HPLC packings.[97] Besides, the advantage of the UPLC method is also related to an increase of the run efficiency and resolution because the particles with 1.7 μm size in UPLC columns allow the chromatographic analysis under much higher pressure and faster flow rate.

MS is a preferable system for the detection of acrylamide among various detectors. However, the disadvantage of MS detection is that the mass of acrylamide itself or its fragment ions is not specific due to the presence of coextractives that yield the same magnitude of m/z with acrylamide. An urgent requirement is the validation of cost-effective, easy-to-use, or widely applicable methods. Some non-MS detection methods have gradually been developed and optimized. For the LC analysis, the ultraviolet (UV)[98] or diode array detection (DAD)[99] at wavelengths of 210 and 225 nm are successfully used for the detection and quantification of acrylamide. Besides, the pulsed electrochemical detection was also reported for the determination of acrylamide.[100] For the GC analysis, electron capture detection (ECD)[68d,76,101] is widely applied because of its high sensitivity. Meanwhile, a recently developed headspace/SPME/GC equipped with a nitrogen−phosphorus detector (NPD) method[102] was validated to analyze acrylamide formed in an aqueous polyacrylamide solution treated by heat or photoirradiation. Besides the LC and GC techniques, the method of thin-layer chromatography (TLC) with fluorescence detection after derivatization with dansulfinic acid[103] was also attractive.

To achieve rapid screening, high-throughput screening, and inexpensive cost, some biological methods were selected for the analysis of large amounts of samples, including a genetic technique[104] and the enzyme-linked immunosorbent assay (ELISA).[105] However, these new methods still need to be optimized, and their analytical results are preferably confirmed by other robust methods.

2.5. Online Determination

To describe the formation of acrylamide during Maillard reaction, changes of precursors, intermediates, acrylamide, and other final products need to be simultaneously concerned. Previous publications reported that MS/MS, proton transfer reaction mass spectrometry (PTR-MS), pyrolysis (Py)-GC-MS, and Fourier transform infrared (FT-IR) spectrometry

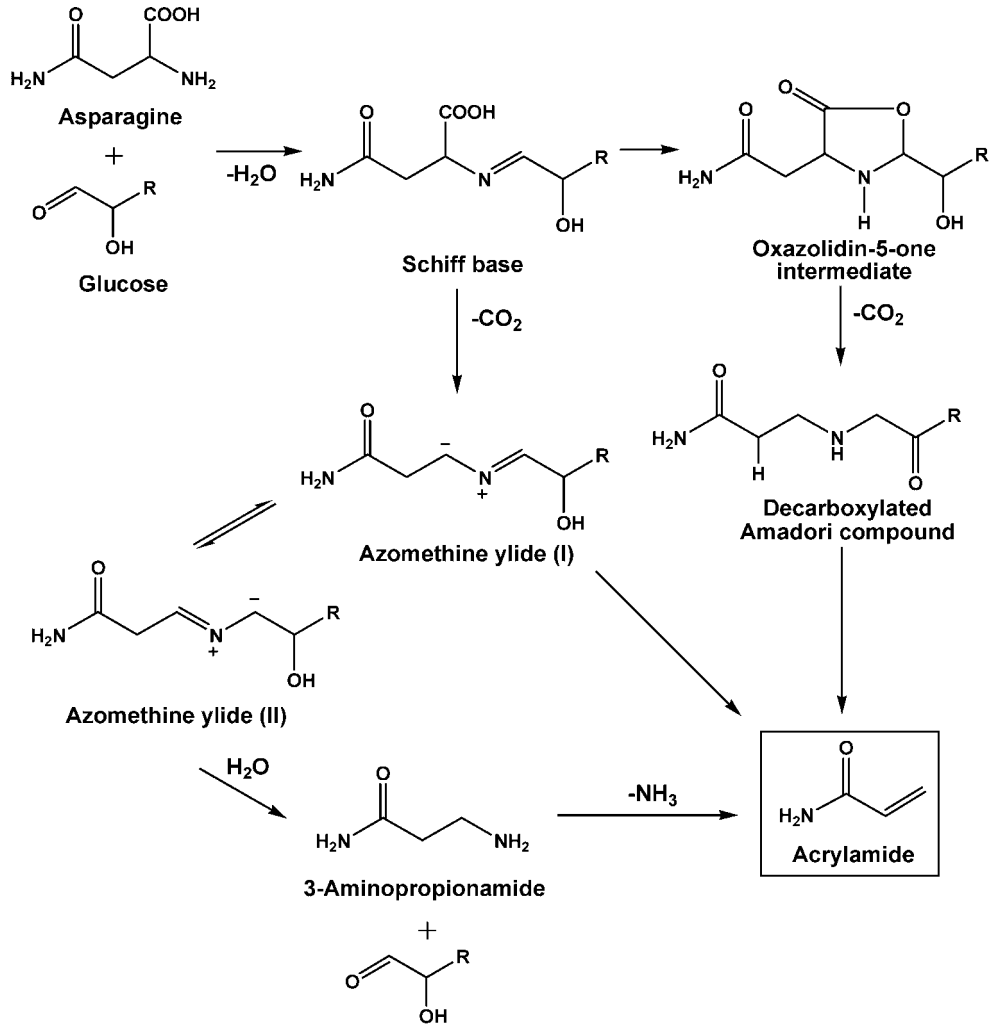

Figure 2. Formation mechanism of acrylamide via the asparagine pathway.

are considered as useful tools to monitor real-time change of acrylamide contents in food matrices or model systems.[106]

Recently, many studies focused on the optimization of different methods for the analysis of acrylamide in various foods. Compared to previous studies since 2002, the goal of analytical studies in 2006−2008 is to improve or establish robust, rapid, easy-to-use, and cost-effective methods. The main points of analytical methods selected during this period are outlined in Table 2. This table supplies pertinent information on the determination of acrylamide, including food matrix, selection of internal standard, pretreatment steps, LOD/LOQ, recovery, and repeatability. These data can be considered as a useful guide for future analytical study on acrylamide in various food matrices.

3. Formation of Acrylamide

3.1. Fundamental Mechanistic Study

3.1.1. Formation Pathway of Acrylamide and Important Intermediates

Shortly after the announcement of the discovery of acrylamide in heat processing foods,[6,47e] numerous research groups in academic schools, industries, and national laboratories all over the world focused on the possible sources and corresponding mechanisms. Publications in *Nature* reported that the main approach of formation of acrylamide in foods is related to the Maillard reaction and especially the amino acid asparagine via water or food matrix model systems.[7] The link of acrylamide to asparagine, which directly provides the backbone chain of the acrylamide molecule, was confirmed by labeling experiments. Mass spectral studies demonstrated that the three carbon atoms and the nitrogen atom of acrylamide are all derived from asparagine. The mechanism of acrylamide formation from a decarboxylated Amadori product of asparagine was shown in Figure 2. In general, some critical and direct precursors contributing to the formation of acrylamide were demonstrated as 3-aminopropionamide, decarboxylated Schiff base,[119] decarboxylated Amadori product,[106c] acrylic acid,[42a,120] and acrolein.[121] On the other hand, lipid oxidation has been suggested as a minor pathway, with acrylic acid as direct precursor formed via acrolein by oxidative degradation of lipids.[53]

3.1.2. Mechanism Elucidation of Asparagine Pathway

As the pathway shown in Figure 2, the first critical step is the amino-carbonyl reaction between asparagine and a carbonyl substance, preferably α-hydroxycarbonyls such as reducing sugars, resulting in the relative *N*-glycosyl conjugation and forming the Schiff base as a key intermediate after dehydration under elevated temperatures. Both the *N*-glycosyl conjugation and the Schiff base are relatively stable under low-moisture systems.[122] In aqueous systems, however, the Schiff base may be hydrolyzed to the precursors or be

Table 2. Some Selected Methods for Determination of Acrylamide in Various Food Matrices

matrix	IS	method	pretreatment	LOD/ LOQ	recovery/ repeatability RSD	ref
coffee and cocoa	$^{13}C_3$-acrylamide	LC-MS/MS, APCI	extraction with water, liquid−liquid extraction with 1:3 ethyl acetate /acetonitrile, SPE with aminopropyl cartridges, Hypercarb column	12.3 μg/kg (LOQ)	0.6−2.5% (repeatability RSD)	Aguas et al.[107]
Brazilian foods	D_3-acrylamide	LC-MS/MS, ESI	extraction with water, SPE with HLB and Bond Elut-Accucat cartridges, μ-Bondapak C_{18} column	10 μg/kg (LOD)	100−115% (recovery)	Arisseto et al.[108]
				20 μg/kg (LOQ)	1.36−8.06% (repeatability RSD)	
coffee	$^{13}C_3$-acrylamide	LC-MS, SIM	defatting with hexane, extraction with water, SPE with Bond Elut Accucat cartridges, derivatization with 2-mercaptobenzoic acid, Merck Lichrosphere 60 Select B column	157 μg/kg (LOD)	~45% (recovery)	Bagdonaite et al.[109]
				270 μg/kg (LOQ)	~5.6% (repeatability RSD)	
various foodstuffs	D_3-acrylamide	FASI-CE-MS/MS, ESI	defatting with hexane, extraction with water, SPE with Strata-X-C and ENV+ cartridges, derivatization with 2-mercaptobenzoic acid	8 μg/kg (LOD)	6.6−15% (repeatability RSD)	Bermudo et al.[90]
				20 μg/kg (LOQ)		
coffee and French fries		LC with pulsed electrochemical detection	filtration, treatment with Carrez I & II, phosphate buffer containing $MgSO_4$, dilution with H_2SO_4 solution, SPE with Strata-X-C cartridges, Synergi Hydro-RP column	1.4 μg/kg (LOD)	86−95% (recovery)	Casella et al.[100]
				3.2 μg/kg (LOQ)		
French fries and chicken legs		GC-MS, SIM	extraction with water, purification with C_{18} cartridges, derivatization with bromination, MDN-5 capillary column	5 μg/kg (LOD)	93% (recovery)	Chuang et al.[110]
various foods	D_3-acrylamide	GC-TOF-MS	extraction with water and *n*-propanol, evaporation, redissolvation with acetonitrile, addition of primary secondary amine sorbent, Innowax capillary column	15−40 μg/kg (LOQ)	97−108% (recovery)	Dunovská et al.[93a]
					~1.9% (repeatability RSD)	
Finnish foodstuffs	D_3-acrylamide	LC-MS/MS, ESI	extraction with water, SPE with HLB cartridges, Hypercarb column	34 μg/kg (LOD)	98−109% (recovery)	Eerola et al.[111]
				68 μg/kg (LOQ)	5−14% (repeatability RSD)	
potato and cereals	D_3-acrylamide	LC-MS/MS, ESI	extraction with water, SPE with HLB and Bond Elut-Accucat cartridges, μ-Bondapak C_{18} column	10 μg/kg (LOD)	100−115% (recovery)	Govaert et al.[112]
				20 μg/kg (LOQ)	1.36−8.06% (repeatability RSD)	
Korean foods	$^{13}C_3$-acrylamide	LC-MS/MS, ESI	extraction with 0.1% formic acid, SPE with HLB cartridges, C_{18} column			Koh[113]
tea	$^{13}C_3$-acrylamide	LC-MS/MS, ESI	rapid pretreatment according to Mastovska and Lehotay,[96] SPE with MCX cartridges, ODS C_{18} column	1 μg/L (LOD)	74−79% (recovery)	Liu et al.[114]
				5 μg/L (LOQ)	1.6−8.3% (repeatability RSD)	
aqueous solution		differential pulse polargraphic (DPP) method		27 μg/L (LOD)	96% (recovery)	Niaz et al.[115]
					0.3% (repeatability RSD)	
water		ELISA	derivatization with 3-mercaptobenzoic acid, incubation, Luna 5 μ C_{18}(2) column	65.7 μg/kg (LOD)		Preston et al.[105a]
chocolate	$^{13}C_3$-acrylamide	LC-MS/MS, ESI	extraction with NaCl, Liquid−liquid extraction with ethyl acetate, SPE with HLB cartridges, Atlantis dC_{18} column	0.3 μg/kg (LOD)	86−93% (recovery)	Ren et al.[68b]
				1 μg/kg (LOQ)	1.6−3.5% (repeatability RSD)	
Spanish foods	$^{13}C_3$-acrylamide	LC-MS, SIM	extraction with water, clarification with Carrez I and II, SPE with Multimode cartridges, Luna ODS2 column	30 μg/kg (LOQ)		Rufian-Henares et al.[116]
Italian foods		GC-MS, SIM	dehydration with anhydrous $NaSO_4$, defatting with hexane, extraction with 2-propanol, no derivatization, Supelcowax-10 fused silica capillary column	25 μg/kg (LOD)	79−85% (recovery)	Tateo et al.[117]
				50 μg/kg (LOQ)		
deep-fried flour-based indigenous Chinese foods		HPLC-UV	extraction with water, SPE with HLB cartridges, detection at 210 and 225 nm, Alltima C_{18} column	6 μg/kg (LOD)	78−107% (recovery)	Wang et al.[98b]
				23 μg/kg (LOQ)	2.1−10.9% (repeatability RSD)	
bakery and potato products	$^{13}C_3$-acrylamide	LC-MS/MS, ESI	LC-MS/MS: extraction with water, SPE with Isolute Multimode and ENV+ cartridges, Hypercarb column		LC-MS/MS: 3.1−7.8% (repeatability RSD)	Wenzl et al.[118]
		GC-MS, SIM	GC-MS: defatting with ethyl acetate and cyclohexane, extraction with water, derivatization with bromination, midpolar capillary column		GC-MS: 5.7−81.9% (repeatability RSD)	
heat-processed starchy foods		GC-ECD	defatting with hexane, extraction with water, derivatization with bromination, liquid−liquid extraction with ethyl acetate, dehydration with anhydrous $NaSO_4$, Supelcowax-10 fused silica capillary column	0.6 μg/kg (LOD)	92.5−101.5% (recovery)	Zhu et al.[101b]
				2.0 μg/kg (LOQ)	3.9−7.1% (repeatability RSD)	

rearranged to the Amadori compound (Figure 2), which is not an efficient precursor during acrylamide formation.[106c] Even under low-moisture conditions, this reaction is most likely the main pathway initiating the early Maillard reaction stage that leads to 1- and 3-deoxyosones, which further decompose, generating color and flavor. This is in accordance with the relatively low transformation yield of asparagine to acrylamide.[19] Alternatively, Schiff base may be apt to make decarboxylation either directly via the Schiff betaine or via the intermediary oxazolidine-5-one to generate the azomethine ylide I, which furnishes the decarboxylated Amadori product after tautomerization.[106c] In summary, acrylamide may be released via the following pathways: (a) directly from azomethine ylide I;[119] (b) via β-elimination reaction from the Maillard intermediate, i.e. the decarboxylated Amadori product;[106c] and (c) via loss of ammonia from 3-aminopropionamide deriving from the azomethine ylide II.[19,123] Such a reaction has been shown to preferentially proceed under aqueous conditions in the absence of sugars.[124]

3.1.3. Acknowledged Parameters Affecting Formation of Acrylamide

At the early stage of the mechanistic study, researchers were concerned about parameters affecting the formation of acrylamide, such as heating time, heating temperature, pH, the ratio of amino acid and reducing sugar, etc. Heating equimolar amounts of asparagine and glucose at 180 °C for 30 min results in the formation of 368 μmol of acrylamide per mol of asparagine.[7b] Adding water to the reaction mixture results in an increase of acrylamide up to 960 μmol/mol. A temperature-dependent study suggested that acrylamide formation also increases with temperature from about 120 to 170 °C and then decreases.[7a] Under similar conditions, methionine is formed in about one-sixth the amount of acrylamide. Similar temperature dependence was observed by Tareke et al.[47e] in laboratory heated foods. Moderate amounts (5−50 μg/kg) were detected in heated protein-rich foods while higher levels (150−4000 μg/kg) were found in carbohydrate-rich foods such as beetroot and potatoes. No acrylamide was found in unheated or boiled foods. However, Ezeji et al.[125] found that acrylamide is formed during the boiling or autoclaving of starch. Other amino acids producing low amounts of acrylamide include alanine, arginine, aspartic acid, cysteine, glutamine, methionine, threonine, and valine.

3.2. New and Further Understanding on Formation Mechanism

3.2.1. Strecker Degradation and N-Glycoside Pathway

Some key intermediates in the asparagine pathway are only predicted, and therefore, the chemical interactions leading to acrylamide remains largely hypothetical. To fill in the gaps under the currently proposed routes, Stadler et al.[126] further demonstrated the mechanism of acrylamide formation in food by comparing the two major hypothetical pathways, i.e. via (i) the Strecker aldehyde route and (ii) glycoconjugates of asparagine. Consequently, they have synthesized appropriate model intermediates and employed these in model systems to obtain a deeper insight into the key precursors and the salient reaction steps. The synthesis of Amadori compounds and *N*-glycosides of amino acids was further demonstrated. At first, Maillard intermediates related to the synthesis of Amadori compounds were prepared via following the general procedure described by López and Gruenwedel,[127] and some key intermediates were identified by NMR,[126,128] which are shown as follows: 2,3:4,5-di-*O*-isopropylidene-β-D-fructopyranose (**1**), 2,3:4,5-(di-*O*-isopropylidene-1-*O*-trifluoromethanesulfonyl)-β-D-fructopyranose (**2**), *tert*-butyl *N*-(2,3:4,5-di-*O*-isopropylidene-1-deoxy-D-fructos-1-yl)-L-asparaginate (**3**), sodium *N*-(2,3:4,5-di-*O*-isopropylidene-1-deoxy-D-fructos-1-yl)-L-asparaginate (**4**), *N*-(1-deoxy-D-fructos-1-yl)-L-asparagine (**5**), *N*-(2,3:4,5-di-*O*-isopropylidene-1-deoxy-D-fructos-1-yl)-benzylamine (**6**), *N*-(2,3:4,5-di-*O*-isopropylidene-1-deoxy-D-fructos-1-yl)-2-phenylethylamine (**7**), and *N*-(1-deoxy-D-fructos-1-yl)-benzylamine (**8**). Second, *N*-glycosides of amino acids were prepared by adapting the general procedure for obtaining *N*-glycosides by condensation of reducing sugars and amino acids in anhydrous methanol under basic pH conditions, first described by Weitzel et al.[129] The main *N*-glucosides include the following: potassium *N*-(D-glucos-1-yl)-L-asparaginate (**9**) and potassium *N*-(D-fructos-2-yl)-L-asparaginate (**10**).

The above analytes **3**−**10** are suitable compounds to study the reaction mechanisms leading to acrylamide under food processing conditions. The experimental results are consistent with the reaction mechanism based on (i) a Strecker type degradation of the Schiff base leading to azomethine ylides, followed by (ii) a β-elimination reaction of the decarboxylated Amadori compound to afford acrylamide. Meanwhile, α-hydroxycarbonyls are much more efficient than α-dicarbonyls in converting asparagine into acrylamide.[126]

3.2.2. Decarboxylated Schiff Base and Decarboxylated Amadori Product

The decarboxylated Schiff base (azomethine ylide) and decarboxylated Amadori product are both key intermediates contributing to the formation of acrylamide (Figure 2). To identify their relative importance, both of the intermediates were synthesized and their relative abilities to generate acrylamide under dry and wet heating conditions were investigated.[130] Under both conditions, the decarboxylated Schiff base [*N*-(D-glucos-1-yl)-3′-aminopropionamide] has the highest intrinsic ability to be converted into acrylamide. In the dry model system, the increase is almost 4-fold higher than that for the corresponding decarboxylated Amadori product or 3′-aminopropionamide. However, in the wet system, the increase was 2-fold higher relative to that for decarboxylated the Amadori product but more than 20-fold higher relative to that for 3′-aminopropionamide.[130]

3.3. Current Model Studies with Certain Matrices

The formation mechanism of acrylamide in asparagine−carbohydrate model systems has almost been clarified via the continuous effort of scientists. But actually, the formation of acrylamide is also greatly related to food matrices, and corresponding parameters on generating acrylamide need to be investigated. Many researchers involve such matrix studies to find the factors affecting acrylamide formed in certain matrices.[33,34,37] The characteristic food matrices include potatoes, bread, cookies, cereal, coffee, almonds, etc. Besides acknowledged parameters, some recently concerned factors, including pretreatment procedures, color development, change of quality attributes, etc., are also gradually attractive.

3.3.1. Potatoes

Potato tubers contain substantial amounts of the acrylamide precursors, including free asparagine, glucose, and fructose, which may explain the high concentrations of acrylamide in certain potato products.[131] French fries, a kind of favorite processed food made of potatoes, are the primary source of acrylamide. Therefore, it is important to reduce concentrations as far as reasonably possible. It is known to us that any modification performed on the raw material constituents will obviously impact the Maillard reaction and its products and, concomitantly, the sensory attributes, including taste and color of the fried food. Small scale and laboratory trials have shown that products such as French fries can be prepared with acrylamide amounts below 100 μg/kg.[132] Furthermore, Fiselier and Grob[133] found that an average concentration of 50 μg/kg acrylamide in French fries could be targeted by limiting the reducing sugars during prefabrication to 0.7 g/kg and the frying temperature to 170 °C. Considering the factor of sensory attributes, the end of the frying process is very critical. A lower temperature toward the end of frying reduces the acrylamide content of the product while color development is still good.[134]

Recently, one of hot topics is the correlation between acrylamide content and color development, i.e. degree of brown as determined by lightness (L^*), redness (a^*), and yellowness (b^*), in potato crisps,[135] low-fat potato snacks,[136] and deep-fried potato products.[137] In general, the acrylamide content in the final potato products should be closely related to the contents of asparagine and sugars. However, Skog et al.[138] indicated that no correlation was found between precursor contents and acrylamide content in home-prepared roasted potatoes. This finding may emphasize the need for further studies on factors affecting acrylamide formation, for example, the availability of precursors at the surface during cooking. Another hot topic is the application of prediction methods to the formation of acrylamide in fried potato products. Based on the experimental values, the formation profile of acrylamide can be predicted via the mathematic models, such as the Logistic-Exponential model, the Empirical model,[139] and heat conduction and mass diffusion equations in Cartesian coordinates.[140] The approach of such model studies can truly compare acrylamide formation profiles and model parameters in the future, with the ability to develop a tool to predict acrylamide formation in potato products.

3.3.2. Bakery Foods

Various kinds of bread and cookies are an important source for considerable amounts of acrylamide in bakery products. The high level of acrylamide in bread is due to a comfortable bakery time, temperature, and precursor levels. Bråthen and Knutsen[141] investigated the starch-based systems, containing freeze-dried rye-based flat bread doughs, flat bread, and bread, which were baked at varying temperatures and times. The formation amount of acrylamide goes through a maximum value at approximately 200 °C, depending on the matrix and the baking time. The amount of acrylamide is reduced at long baking time. However, the amount of acrylamide in bread crust increases with both baking time and temperature.

A characteristic source for high amounts of acrylamide, gingerbread, is found and demonstrated in some European countries. Konings et al.[142] measured acrylamide in Dutch gingerbread products ranging from 260 to 1410 μg/kg. It is concluded that the acrylamide level in gingerbread can be significantly mitigated by using hydrogencarbonate as baking agent, minimizing free asparagine, and avoiding prolonged baking.[143] During these years, many studies investigated the factors affecting the formation of acrylamide in fermentation bakery products, such as yeast-leavened bread, wheat bread and bread rolls, cheese bread, etc. For the yeast-leavened bread, addition of asparagine strongly increases acrylamide content, while addition of glycine decreases the content.[144] For wheat bread and bread rolls, factors including addition of cysteine to the dough, increased fermentation time, reduced baking temperature, and prolonged heat treatment are in favor of controlling the acrylamide content.[145] For cheese bread, acrylamide was not observed in a traditional Brazilian bread product made from fermented cassava flour, fresh eggs, and a mixture of Brazilian Gouda type cheese and Mozzarella cheese, pointing toward a role of eggs in protection against acrylamide formation.[146] Another important source is cookies. Studies demonstrated that the pH of the dough formula, the content of reducing sugars,[147] and the browning ratio of products[148] are significantly related to the formation of acrylamide in cookies.

3.3.3. Cereal

Cereal-deriving foods become another key source for the formation of acrylamide during heat processing. Wheat and rye are two important cereal matrices for daily staple foods in some Western countries.[21] Springer et al.[149] showed that free asparagine levels varies across the rye grain, with the lowest level in the endosperm and the highest level in the bran. Acrylamide levels in wafers made from rye flours reflects their free asparagine content, with acrylamide levels in bran being greater than those in cooked wholemeal rye, which are greater than those in cooked rye flour without bran. Elmore et al.[150] studied the relationship between acrylamide and its precursors in wheat and rye. Between 5 and 20 min, major losses of asparagine, water, and total reducing sugars are accompanied by large increases in acrylamide, which are maximized between 25 and 30 min, followed by a slow linear reduction when the cereal matrices are cooked at 180 °C. Acrylamide formation does not occur to a large degree until the moisture content of the cakes falls below 5%. A significant finding for commercial rye flours was that asparagine levels are correlated with fructose and glucose. The results suggested that, for these commercial cereals, selection based on low fructose and glucose contents, and hence low asparagine, could be beneficial in the mitigation of acrylamide.[151] Besides the factor of precursor contents, recent topics are interested in the effect of a suitable cultivar on the change of precursors and consequently the formation of acrylamide during heat processing. A considerable effect of sulfur nutrition during the cultivar period on the generation of acrylamide in cooked wheat was observed.[152] When wheat was grown under conditions of severe sulfate depletion, dramatic increases in the concentration of free asparagine were found and thus unexpected high levels of acrylamide were present.[153] Furthermore, the impact of nitrogen amounts[154] and different nitrogen fertilizers[155] on the quality and potential of acrylamide formation in winter wheat should also be taken into consideration.

3.3.4. Coffee

Coffee is usually roasted at temperatures in the range of 220−250 °C. Coffee beans are processed at relatively higher temperatures than those for other foods, and therefore the reaction and formation of acrylamide during roasting are more complex than those for other matrices. Two research groups have found that acrylamide is not stable in commercial coffee stored in its original container.[52,55a] Losses of 40% to 60% have been recorded in ground coffees stored at room temperature. Acrylamide in coffee is formed at the very beginning of the roasting step, reaching more than 7 mg/kg and then declining sharply toward the end of the roasting cycle due to high rates of elimination.[34] Increasing the roasting degree leads to a decrease in acrylamide concentration as well as radical scavenging capacity.[156] Nevertheless, deep roasting as a potential choice to mitigate acrylamide could generate other undesirable compounds and negatively impact the sensory properties of the product. In view of all possible changes of acrylamide content, its formation and elimination during roasting, storage, and brewing need to be sequentially considered. Lantz et al.[157] systematically showed that the main factors affecting the level of acrylamide in roasted coffee appear to be the Arabica/Robusta ratio (with Robusta giving higher levels), time and degree of roast (with both shorter and lighter roasting at the edges of the normal roasting range giving higher levels), and storage conditions and time (with clear reduction at ambient storage). Study of ^{14}C-labeled acrylamide as radiotracer found that no formation of volatile ^{14}C-acrylamide-related compounds was detected during storage and coffee brewing. Close to 90% of the radiolabel in the filter residue (spent roasted and ground coffee, spent grounds) remains firmly bound to the matrix.[158]

3.3.5. Almonds

Almonds also contain both acrylamide precursors in appreciable levels. The content of free asparagine was reported in the range of 2000−3000 mg/kg[159] while glucose and fructose levels were analyzed to be 500−1300 mg/kg, and sucrose contents ranged from 2500 to 5300 mg/kg.[160] As a result, the detection of acrylamide in roasted almonds in concentrations from 260 to 1530 μg/kg[42a] was not surprising. Interestingly, acrylamide was found to decrease in roasted almonds during storage at room temperature.[131] The formation of acrylamide in almonds starts only when the kernel temperature exceeds approximately 130 °C. The color as measured by the degree of brightness correlates well with the acrylamide content, as acrylamide content increases with increasing darkness. At constant roasting conditions, almonds with higher initial moisture content contain less acrylamide after roasting, which is probably due to the influence of moisture on the development product temperature during roasting.[161]

3.3.6. Olives

Black ripe olives are one of the most important classes of table olive commercialized in the world.[162] Acrylamide levels in black ripe olives are affected by variations in processing conditions at the different manufacturers, as well as in different forms of olive presentation (sliced, pitted, or chopped) by the same manufacturer.[163] The effects of darkening method and olive cultivar on the acrylamide content were pronounced as the key factors. Acrylamide contents do not significantly differ after 6 months of storage. The small amounts of free amino acids and reducing sugars found in olives before sterilization do not significantly correlate with the acrylamide formed.[164]

3.4. New Developments on Impact Factors

3.4.1. Research Progress on Acknowledged Factors

The formation of acrylamide is affected by multiple factors. At the early stage of studies, researchers focused on the parameters, such as heating time, heating temperature, the ratio of amino acid and reducing sugar, etc. Initial studies were almost performed in model systems with pure chemicals, such as the asparagine−glucose model system. Heating equimolar amounts of asparagine and glucose at 180 °C for 30 min resulted in the formation of 368 μmol of acrylamide per mol of asparagine.[7b] A temperature-dependent study suggested that acrylamide formation also increases with temperature from about 120 to 170 °C and then decreases.[7a]

Compared to model systems, the formation of acrylamide in actual food matrices produced via various heat processing methods, such as frying, baking, and roasting, is more complex. Meanwhile, the control of acrylamide content and maintenance of original food quality need to be simultaneously considered during heat processing. Fiselier et al.[165] recently summarized the effect of frying temperature on the formation of acrylamide and demonstrated that the temperature during the second half of the process is more important than that regulated by the thermostat since acrylamide is formed toward the end of frying. Optimized fryers should allow an initial temperature drop but then efficiently heat to prevent the temperature from dropping below a given limit; after the end of frying, the initial temperature must be restored before frying the next portion. Romani et al.[166] indicated that the increase of frying time becomes a key factor in terms of the quantity of acrylamide and its formation rate when the temperatures of the potato surface and the oil bath reach 120 and 140 °C, respectively, after around 4 min of frying.

Besides, other factors related to the formation of acrylamide include precursor levels and water content in raw materials, pretreatment, pH, etc. The contents of precursors, including asparagine, glucose, and fructose, play an important role in the generation of acrylamide. Since the asparagine content is approved as a prerequisite for the heat-induced formation of acrylamide, control of free asparagine could turn out to be a useful approach to mitigate acrylamide formation. Changes in asparagine content appear to be a good indicator of changes in nitrogen metabolism of plants induced by pesticides and environmental factors.[24] A direct relationship between the acrylamide contents and asparagine levels was demonstrated in baked/toasted wheat and rye breads.[167] The reducing sugars glucose and fructose have also been reported to serve as important contributors.[168] Recently, a function of different glucose/fructose ratios in raw potato products using several color measurement methods was optimized to investigate the relationship between acrylamide content and Maillard browning in French fries.[169] Based on current findings, acrylamide is formed in different amounts with several mono- or disaccharides. Even nonreducing sugars, such as sucrose, are efficient reactants, leading to the release of reducing sugars that are then available to react with the α-NH_2 group of asparagine via the Maillard pathway after thermally induced hydrolysis.[33,37] Some other factors

such as water content and pretreatments also need to be indicated. Crust temperature in combination with water content has a significant effect on acrylamide formation during the baking of white bread. Acrylamide concentration was observed to decrease at very high temperatures and lower water contents.[170] For the effect of pretreatments, blanching and addition of sodium solutions before frying are usually concerned. The initial report indicated no effect of blanching as pretreatment on the concentration of acrylamide in the potato crisps.[171] However, current research further optimized the pretreatment methods and found many details. Soaking of blanched potato slices in the 3 g/100 g of NaCl solution per 5 min at 25 °C reduces acrylamide formation in potato chips by 11% after frying at 170 °C. However, when the slices are blanched directly in the 3 g/100 g of NaCl solution at 60 °C for 30 min, their acrylamide formation increases surprisingly by ~90% when frying at 170 °C.[172] To elucidate the effect of NaCl, Mestdagh et al.[173] indicated the acids, NaCl, and calcium-containing additives lower the oil absorption, which may lead to a reduced heat transfer and acrylamide contamination in the final product. Besides, several studies[120b,174] have demonstrated that pH modification is a potential way to control the formation of acrylamide in food and model systems. Basic pH conditions are in favor of the release of acrylamide during poststorage.[130]

Besides the above routine factors, it is acknowledged that the formation of acrylamide is also related to other minor factors, some of which are highlighted recently and described in the following sections.

3.4.2. Agronomic Factors during Cultivation of Raw Materials

The agronomic factors mainly include fertilization methods, harvest season, and climatic conditions. A reverse correlation between the amount of fertilizer applied in potato cultivation and the acrylamide content in the edible products has been revealed, since reducing sugar contents are elevated while crude protein and free amino acids decrease when less nitrogen-fertilizer is given.[175] Gerendás et al.[176] observed the same phenomenon and confirmed that the highest acrylamide contents were observed in tubers grown with high nitrogen and inadequate potassium supply, which also contain the highest contents of precursors. Furthermore, the influence of sulfur fertilization should be considered. When wheat was grown under conditions of severe sulfate depletion or sulfur deficiency during fertilization, dramatic increases in the concentration of free asparagine were found and subsequently enhancement of acrylamide content during baking was observed.[153,177] Independent of fertilization, harvest year and climatic conditions turn out to be other factors influencing acrylamide formation. Favorable light and temperature conditions during the cultivation period enhance amino acid and protein contents, thus promote the formation of acrylamide during baking.[178]

3.4.3. Variety and Storage Conditions of Raw Materials

Acrylamide formation in crisps can be reduced by using potato varieties with low levels of both asparagine and reducing sugars. Mass transport of precursors during heating is suggested to be important for acrylamide formation in potato crisps.[179] Many researchers[132,180] demonstrated that the acrylamide level in potato chips made from tubers stored at low temperature is much higher than that from those stored at high temperature. It seems that storage of raw materials at low temperatures should be avoided for the control of precursors. However, storage at too high a temperature can reduce the preservation period and sensory attributes of raw materials. To cope with this problem, Paleologos and Kontominas[181] observed a minimum value of acrylamide concentration when the breaded chicken products are stored under refrigeration with a modified atmosphere mixture of 60% CO_2 plus 40% N_2. Overall, the effect of variety and storage conditions of raw materials is ascribed to the variation of amino acids and reducing sugars.

3.4.4. Change or Modification of Heat Processing Methods

Blanching instead of frying or soaking before frying or blanching could significantly affect the acrylamide level.[171,182] However, such an idea seems not very practical because the negative effects on the connatural and sensory characteristics of processing foods may inevitably occur. To effectively control the formation of acrylamide, some tips regarding heat processing methods were recommended such as low temperature vacuum frying,[183] short time heating, and avoiding the use of palm olein as for modification of processing. Recently, the microwave heating method is highlighted. Microwave heating as a fast and convenient heat processing method is widely applied all over the world. Microwave heating provides a favorable medium for the occurrence of acrylamide and probably affects the formation and kinetics of acrylamide distinguishingly due to its extraordinary heating style. Yuan et al.[184] demonstrated that the acrylamide content dramatically increases in potato chips by microwave treatment, which is significantly higher than that produced by traditional frying. Zhang et al.[185] systematically investigated and compared the formation of acrylamide in asparagine-sugar microwave heating model systems, which indicated that microwave heating is a potential source for the formation of large amounts of acrylamide. However, microwave frying also can be an alternative for the generation of low levels of acrylamide if the frying time is effectively limited.[186]

3.4.5. Correlation with Color Development

Color not only is visually considered one of the most important parameters in the definition of quality of fried products but also is the result of the Maillard reaction, which depends on the content of reducing sugars and amino acids or proteins at the surface, and the temperature and time of frying.[187] A direct correlation between the acrylamide generation and color development of products was approved in many studies.[136,161,187,188] Meanwhile, the acrylamide generation in products treated by various improved pretreatment methods can also be observed via the change of color kinetics.[189]

Compared to previous studies since 2002, the goal of formation studies in 2006−2008 is to find new factors and improve acknowledged factors contributing to the formation of acrylamide. Besides the description of the above research progress, the main points of formation studies selected during this period are highlighted in Table 3.

Table 3. Some of Recent Studies and Findings on Formation of Acrylamide in Various Food Matrices

sample matrix	main findings	ref
potatoes, almonds, bakery products, olives, and dried fruit	In potatoes, the control of reducing sugars, process temperature, and moisture is imperative to limit acrylamide formation. In bakery products, free asparagine and the type of baking agent (NH_4HCO_3 or $NaHCO_3$) largely determine acrylamide formation and present the starting points for reduction. Temperature and free asparagine are the key factors for acrylamide formation in roasted almonds.	Amrein et al.[190]
bakery products	The influence of radiofrequency heating on acrylamide formation was studied.	Anese et al.[191]
lyophilized peptide	The formation of acrylamide from asparagine and β-alanine containing peptides was described.	Buhlert et al.[192]
potato products	The addition of enzyme (2 U/g) and the following incubation of the mixture at 37 °C for 30 min lead to 70% reduction of acrylamide content.	Ciesarová et al.[193]
potatoes and wheat	The water content is one of the most important factors in the formation of acrylamide. Fructose is more effective for the acrylamide formation in comparison with glucose.	Ciesarová et al.[194]
purified wheat gluten and wheat bread rolls	Contents of asparagine and reducing sugars are diminished due to the addition of the gluten. The increase in acrylamide is significant when comparing 0 and 15% gluten addition.	Claus et al.[195]
fried potato crisps	A farm-to-fork human exposure assessment model for acrylamide for Irish consumers was developed.	Cummins et al.[196]
potatoes	A significant impact of variable climatological conditions on the reducing sugar, dry matter, total free amino acid, and free asparagine contents of tubers was observed. Warm summers give rise to a lower reducing sugar content and thus a lower susceptibility to acrylamide generation during frying.	De Meulenaer et al.[197]
cooked potatoes	In potatoes, where concentrations of sugars are usually limiting, competition between asparagine and other amino acids participating in the Maillard reaction may be a key determinant of the amount of acrylamide that is formed during processing.	Elmore et al.[198]
French fries	The acrylamide content of the surface is found to be 72, 2747, and 6476 μg/kg after frying for 9 min at 150, 170, and 190 °C, respectively. The core is free of acrylamide after frying for 9 min at 150 and 170 °C, while only 376 μg/kg of acrylamide is formed at 190 °C.	Gökmen et al.[199]
asparagine-glucose model system	Strong evidence showed that the cations effectively prevent the formation of Schiff base and mainly change the reaction path toward glucose dehydration, leading to hydroxymethylfurfural and furfural.	Gökmen and Şenyuva[200]
potatoes and rye flour	Strongly alkaline conditions seem to induce net formation of acrylamide from water-soluble precursors formed during thermolysis.	Goldmann et al.[201]
asparagine-glucose aqueous glycerol system	Acrylamide formation from asparagine and glucose in different ratios in neutral glycerol/water mixtures increases with decreasing water activity ($0.33 \leq a_w \leq 0.71$ investigated) and increasing temperature (120 °C $\leq T$ 160 °C investigated).	Hedegaard et al.[202]
low-moisture starch-based model systems	Highly significant correlations were obtained for the relationship between pyrazine and acrylamide formation.	Koutsidis et al.[203]
fried silica gel model system	The contribution of acrolein to the overall formation of acrylamide appears to be negligible in the presence of a reducing sugar, indicating that in foodstuffs the importance of acrolein and other oil degradation products is probably small.	Mestdagh et al.[204]
potato powder model system	The acrylamide content is rather dependent upon the moisture content than upon the water activity in the high-moisture potato powder model system.	Mestdagh et al.[205]
French fries	The investigated oil degradation products, such as glycerol and mono- and diacylglycerols, do not significantly influence the acrylamide formation.	Mestdagh et al.[206]
green tea	Roasting at 160 °C is recommended for Houjicha processing for acrylamide mitigation, formation of potent odorants, and suppression of degradation of tea catechins.	Mizukami et al.[207]
French fries	Soaking of blanched potato strips (75 °C, 10 min) in an 10000 U/L asparaginase solution at 40 °C for 20 min is an effective way to reduce acrylamide formation.	Pedreschi et al.[208]
roots, tubers, and plantain products	Roots and tuber products have relatively high nonenzymatic browning and acrylamide levels, while plantain products show low levels of nonenzymatic browning and acrylamide.	Quayson and Ayernor[209]
potato chips	The artificial neural network (ANN) modeling approach was shown to successfully predict acrylamide concentration and browning ratio of potato chips during frying in a time-dependent manner.	Serpen and Gökmen[210]
model cookies	A direct correlation was found between acrylamide levels and the antioxidant activity of model cookies	Summa et al.[211]
potato products	Impact of harvest year and food matrix on the generation of acrylamide was observed.	Viklund et al.[212]
aqueous polyacryl-amide/iron mixtures	At acid/neutral pH, the amount of acrylamide released is related to the concentration of ferric ion and the irradiation time. At alkaline pH, polyacrylamide/Fe^{3+} mixtures are stable under irradiation.	Woodrow et al.[213]
asparagine-glucose/fructose model system	High correlation of methylglyoxal with acrylamide formation in model systems was demonstrated.	Yuan et al.[214]
model systems	Both unoxidized and oxidized lipids are able to contribute to the conversion of asparagine into acrylamide, but unoxidized lipids need to be oxidized as a preliminary step.	Zamora and Hidalgo[215]
low-moisture asparagine-sugar model systems	Acrylamide is readily formed via heating binary precursors for 15 min at 180 °C in the glucose and fructose system while acrylamide was readily generated when the binary precursors are heated for 15 min at 210 °C in the sucrose system.	Zhang et al.[216]

3.5. Kinetics of Acrylamide Generation and Elimination

The kinetics of acrylamide generation and elimination in heat processing foods has been comprehensively elucidated. Currently, kinetic models established in model systems or food matrices include the first-order formation/elimination model,[217] the mechanistic model,[82] and the nonisothermal model.[22]

The first-order formation/elimination models, which include the first-order kinetic model, the logistic-Fermi model, and the logistic-exponential model, are mostly used for the kinetic studies. For the first-order kinetic model, the acrylamide contents calculated can be regarded as the net result of consecutive formation and elimination reactions.[217] The whole kinetics of acrylamide content can be formulated by a simplified reaction schedule of two simultaneous reactions in which k_F and k_E represent the rate constants of acrylamide formation and elimination processes, respectively. The kinetic equations are listed as follows:

$$\frac{dC}{dt} = -k_F C \quad (1)$$

$$\frac{dC_{AA}}{dt} = k_F C - k_E C_{AA} \quad (2)$$

$$\frac{dC_D}{dt} = k_E C_{AA} \quad (3)$$

Among eqs 1−3, C, C_{AA}, and C_D represent the concentrations of asparagine/glucose, acrylamide, and degradation/elimination products, respectively, while t represents the reaction time. Similarly, such a first-order kinetic model was established in the asparagine−fructose system.[218] Using this model, Claeys et al.[219] investigated the effect of four amino acids (glutamine, cysteine, lysine, and alanine) on the kinetics of acrylamide and found the ratio of the elimination to the formation rate constant significantly increases, indicating the competitive mitigation effect of four amino acids on the reaction between asparagine and glucose. Recently, kinetic-related studies also observed the effect of pH, water activity, different moisture levels, and different initial reactant concentrations and ratios on the kinetic behavior of acrylamide in aqueous or potato-based asparagine-glucose systems.[220] For the logistic-Fermi model, when considering the two main processes, i.e. generation and degradation, the isothermal concentration $C(t)$ of acrylamide can be fitted as the product of these two functions, i.e. $C_g(t)$ and $C_d(t)$ (eq 4). In detail, $C_g(t)$ follows the shifted logistic function while $C_d(t)$ follows the Fermi distribution function, which is the mirror profile of the logistic function.[22,221]

$$C(t) = C_g(t)\, C_d(t) \quad (4)$$

$$C_g(t) = \frac{a}{1 + \exp[k_g(t_{cg} - t)]} - \frac{a}{1 + \exp(k_g t_{cg})} \quad (5)$$

$$C_d(t) = \frac{1}{1 + \exp[k_d(t - t_{cd})]} \quad (6)$$

Among eqs 4−6, a, k_g, and t_{cg} are temperature dependent coefficients during acrylamide generation while k_d and t_{cd} are temperature dependent coefficients during acrylamide degradation. For the logistic-exponential model, the acrylamide degradation process is described as a simple exponential function (eq 7) and the acrylamide concentration $C(t)$ can also be fitted as the product of $C_g(t)$ and $C_d(t)$. The only difference between the logistic-Fermi model and the logistic-exponential model is the discrepancy of the $C_d(t)$ function expression.[22]

$$C_d(t) = \exp\left(-\frac{t}{\tau}\right) \quad (7)$$

In eq 7, τ is a characteristic time.

The mechanistic model clearly describes the details of the kinetics of precursors, intermediates, acrylamide, degradation products, and other Maillard reaction products. For the kinetic rate constants, k_1, k_2, k_3, k_4, k_5, and k_6 are expressed as loss of asparagine and glucose, formation of fructose, loss of asparagine and fructose, formation of acrylamide, formation of melanoidins, and elimination of acrylamide, respectively. For each reaction step, a differential equation was set up by the use of the law of mass action, and the obtained differential equations were solved by numerical integration.[82] Based on the above parameter designs, the kinetics of acrylamide and Schiff base intermediates could then be simultaneously studied though Schiff base intermediates and possibly also instantaneous compounds during the whole Maillard reactions.[222]

Compared to the above two kinetic models, the nonisothermal model has rarely been applied because the temperature variation rate should be additionally considered. Kolek et al.[223] investigated the effect of table salt on the elimination of acylamide using the nonisothermal model.

4. Mitigation Recipes of Acrylamide

4.1. Mitigation Mechanism of Acrylamide

According to current research progress, possible mitigation pathways can be considered in the following ways: (i) mitigating the formation and transformation of some key intermediates via modifying reaction conditions; (ii) controlling reaction conditions in order to preferably form some other small molecules during the final stage of the reaction; (iii) inhibiting the key procedures in the Maillard reaction, such as the formation of Schiff base, Strecker degradation, the *N*-glycoside pathway, and the β-elimination reaction of decarboxylated Amadori products.

Mechanistic study demonstrated that three other final products including maleimide, succinimide, and niacinamide may be generated besides the formation of acrylamide during the asparagine pathway.[106c] Thus, acrylamide can be controlled via inducing the reaction toward the formation of these three products. First, the mitigation of acrylamide can be achieved via the control of decarboxylation of asparagine. Evidently, intramolecular cyclization to form the 3-aminosuccinimide intermediate is much faster compared with the decarboxylation reaction due to the entropy factor.[224] Thus, the formation of maleimide is preferably achieved. Second, the mitigation of acrylamide can be achieved via the control of the Schiff form of *N*-glycosylasparagine, which can stabilize itself through intramolecular cyclization initiated by the carboxylate anion.[225] Thus, the formation of oxazolidin-5-one is subsequently reduced. The Schiff form of *N*-glycosylasparagine can generate Amadori product through Amadori rearrangement, which prevents the Schiff base from

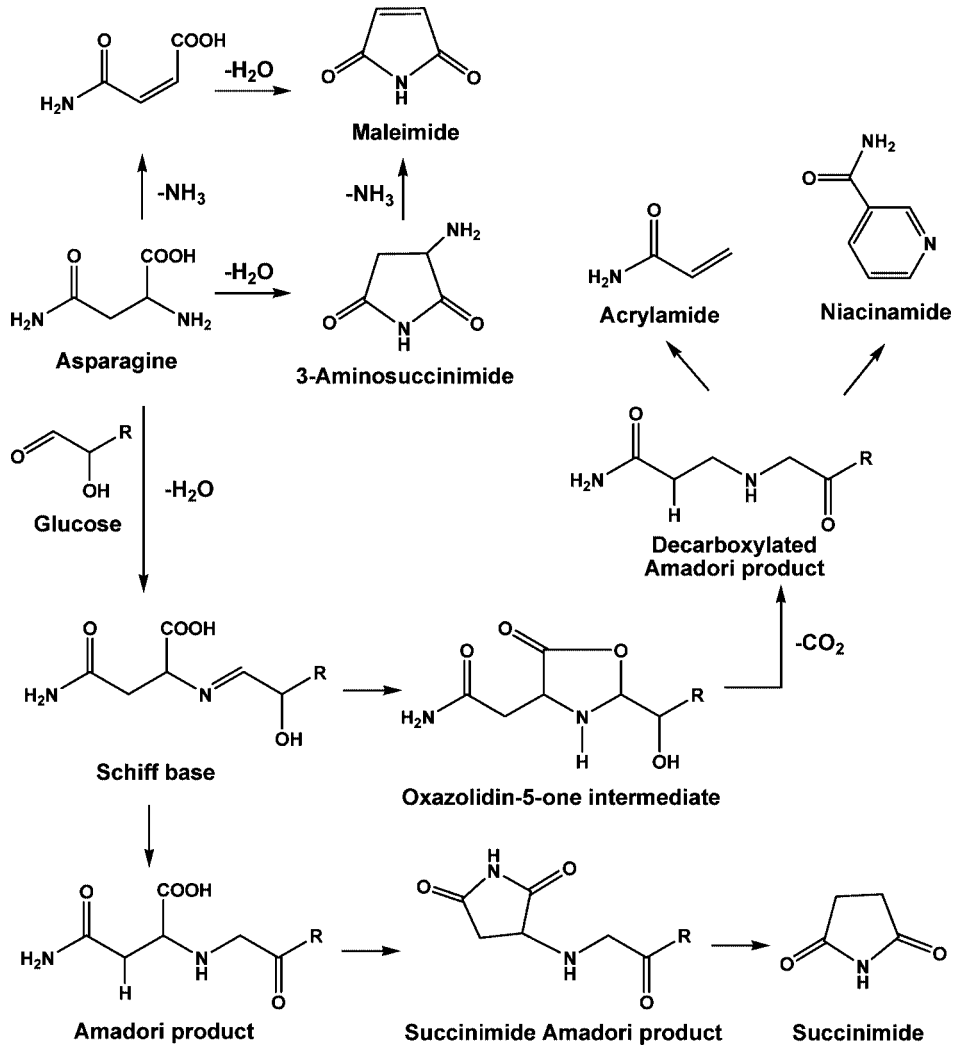

Figure 3. Formation mechanism of maleimide, succinimide, and niacinamide.

generating decarboxylated Amadori product through intramolecular cyclization and decarboxylation. Subsequently, the Amadori product can generate the succinimide Amadori intermediate and further lead to the formation of succinimide.[106c] Third, the preferable formation of niacinamide can competitively reduce the generation of acrylamide. The formation mechanism of maleimide, succinimide, and niacinamide during the asparagine pathway is shown in Figure 3.

4.2. Current Progress on Acknowledged Mitigation Recipes

Recently, some published review articles reported the reduction methods of acrylamide in potatoes,[226] cereal products,[28] coffee,[227] etc. The acknowledged mitigation recipes mainly include the modification of raw materials and optimization of the heat processing parameters, such as change of precursors in raw meterials, optimization of heat processing methods and parameters, modification of cultivar techniques, storage temperature of materials, fermentation, etc.

4.2.1. Control of Asparagine and Reducing Sugar Contents

Previous study[133] found that a suitable limit for the reducing sugars during prefabricating is a simple and efficient approach to reduce the exposure to acrylamide from the predominant source for many consumers. The acrylamide level in potato chips made from tubers stored at low temperature (<10 °C) is much higher than that stored at high temperature (>10 °C) based on the consideration of storage conditions.[132,180c] Thus, an appropriate storage temperature for the raw materials is required. Meanwhile, the proper fertilization of nitrogen and potassium is in favor of the control of asparagine contents in foodstuffs and the reduction of high amounts of acrylamide during heat processing.[228] Therefore, the following tips should be concerned when the control of precursor contents is used for the limitation of acrylamide concentrations: (i) choice of plant cultivars low in either asparagine or reducing sugars; (ii) avoidance of storage at too low temperatures; (iii) optimization of the blanching process to extract the maximum amount of sugar and asparagine; (iv) avoidance of adding reducing sugars in further treatments.

4.2.2. Optimization of Heat Processing Parameters or Methods

The control of important processing parameters, which include heating temperature, heating time, oil type, etc., could be regarded as the most direct way to mitigate acrylamide. The evidence indicated that there is not a simple linear increase between the acrylamide level and heating temper-

ature, but the acrylamide concentrations are noticed to increase linearly with heating time.[7a] The selection of appropriate heating temperature and avoidance of too long heating time are in favor of controlling the formation of large amounts of acrylamide. For the effect of heating temperature, recent findings indicated that acrylamide levels in French fries can be mitigated by half if the final stage of the frying process employs a lower oil temperature.[229] For the effect of oil type, the origin of the deep-frying vegetable oils do not seem to affect the acrylamide formation in potatoes during frying.[230] However, the formation of acrylamide in food is much higher when using palm olein or frying oils containing silicone.[53]

Based on the above contributions, current studies specially focus on the optimization of pretreatments and heat processing methods. For the modification of pretreatments, additional immersion[231] and blanching[182,232] procedures before frying could significantly mitigate the acrylamide levels. Such a mitigation effect may be partly ascribed to the loss of precursors in original materials during pretreatments. For the improvements of heat processing methods, the mitigation of acrylamide may be achieved by low temperature heating such as low temperature vacuum frying. Besides, Erdőgdu et al.[233] found that when the potato strips are subjected to frying after a microwave precooking step, the acrylamide content in the whole potato strip is reduced by 36%, 41%, and 60% for frying at 150, 170, and 190 °C, respectively, in comparison to the control. Therefore, the vacuum frying and microwave precooking techniques are highlighted as potentially effective methods for the mitigation of acrylamide. However, the original sensory attributes of foods should be preferably concerned and guaranteed when using these improved methods during practical heat processing. Besides the above-mentioned reducing methods, the elimination of acrylamide could also be achieved via lactic acid fermentation,[234] use of asparaginase,[235] or coating with egg/breadcrumbs.[236]

4.3. New and Improved Mitigation Recipes

Based on the maintenance of original sensory attributes and heat processing procedures, the use of exogenous additives is highlighted as an effective mitigation recipe in recent studies. These exogenous additives include food additives, food formula, and plant extracts. Such a mitigation recipe should comply with the following conditions: (i) The addition level should be properly controlled according to corresponding criteria of food or chemical additives. (ii) The selected additives should be regarded, as no toxicity was demonstrated by toxicity tests from previous publications. (iii) The additives applied to the food systems cannot affect the connatural and sensory characteristics of processing foods.[37] During these years, many additives have been found to have the inhibitory effect of acrylamide formation in the Maillard reaction, as well as the addition levels and methods are also improved. Meanwhile, the irradiation and genetic modification techniques are gradually developed for the mitigation of acrylamide.

4.3.1. Addition of pH Modifier

Previous studies[174] have demonstrated that pH modification is an effective way in which to reduce acrylamide formation in food and model systems. The acrylamide level can be effectively mitigated via lowering the pH of the food system. Such a change may attribute to protonating the α-amino group of asparagine, which subsequently cannot engage in nucleophilic addition reactions with carbonyl sources.[174b] Citric acid is a representative compound for the modification of pH, which is approved as an effective acrylamide inhibitor.[237] Cook and Taylor[174a] reported that the effect of citric acid addition alone is a 23.5% reduction in acrylamide at pH 4.48 and 47% reduction at pH 3.93. Such reduction rates are less in percentage terms than those reported by Jung et al.[174b] when modifying the pH of corn grits (also with citric acid) prior to frying. The correlation between pH decrease and acrylamide reduction may have differences among products, due to multiple factors or different starting pH values of the products. Besides, the largest decrease of acrylamide content (90%) in crisps was observed when potato slices were soaked in acetic acid solution for 60 min at 20 °C.[238] Although acetic acid can also be used for the mitigation of acrylamide, the sensory attributes of the food matrix seem unacceptable because of the volatile odor of acetic acid. Therefore, the use of acetic acid is not recommended.

4.3.2. Addition of Proteins or Amino Acids

Previous *in vitro* study demonstrated the reduction of acrylamide uptake by dietary proteins in the Caco-2 gut model.[239] The acrylamide molecule contains an active double bond, which may interact with food ingredients, mainly proteins, DNA, and RNA. *In vitro* incubation of acrylamide together with glutathione (GSH) at pH 8 significantly reduces the amount of acrylamide monomers to 81%. Meanwhile, a higher availability of cysteines (molar ratio 1:10; acrylamide/GSH) leads to 48% reduction of acrylamide because acrylamide is most likely bound covalently to glutathione via Michael addition of cysteine residues to the terminal double bond.[239] Similar results have been demonstrated via *in vitro* scavenging reactions of acrylamide with GSH.[240] The degradation mechanism of acylamide *in vitro* by GSH is mainly via the decomposition of the glycine fragment of GSH. The glycine moiety can be eliminated more readily by the cleavage of peptide bonding in the presence of acrylamide. The glycine in the solution is degraded by the Strecker degradation to aldehyde, ammonia, and carbon dioxide. This degradation product, such as formaldehyde, reacts with acrylamide, which leads to decomposition of acrylamide to small molecular fragments. Then, acrylamide can be transformed to acrylate, which is susceptible to consecutive decarboxylation and further total oxidation by the catalytic processes of degraded products of glycine.[240] This scavenging reaction is similar to the catalytic total oxidation of acrylamide in the presence of water via the formation of acrylate.[241] For plant-derived proteins, soy protein hydrolysates can also be used to mitigate the formation of acrylamide because they are believed to reduce acrylamide by introducing additional amino acids to compete with asparagine for key reaction intermediates.[242]

4.3.3. Addition of Hydrogencarbonates

The mitigation effect on the formation of acrylamide by hydrogencarbonates is complex.[143] Studies on elimination and net formation of acrylamide caused by sodium hydrogencarbonate ($NaHCO_3$) showed that three different addition levels have a similar effect on elimination. Net acrylamide increases somewhat as bicarbonate drops to near 1% but remains significantly suppressed compared with the control sample. Potassium hydrogencarbonate ($KHCO_3$) has an

inhibitory effect similar to that of the sodium compound except that net formation appears to be slightly higher. Conversely, ammonium hydrogencarbonate (NH_4HCO_3) with the same addition levels as $NaHCO_3$ and $KHCO_3$ could dramatically enhance the acrylamide concentration.[243] The interpretation of great differences among three bicarbonates is also complicated by the change in the temperature curve known to accompany bicarbonate addition. Recently, researchers found that removing ammonium-based raising agents is beneficial for the mitigation of acrylamide in biscuits.[244]

4.3.4. Addition of Mono- or Divalent Cations

The mitigation effect of mono- or divalent cations on the generation of acrylamide is also concerned. Kolek et al.[245] proved considerable inhibiting effects of sodium chloride (NaCl) on the formation of acrylamide in mixtures of asparagine−glucose model systems. Compared to the systems without NaCl addition, the acrylamide contents were lowered roughly by 32%, 36%, and 40% when the addition levels of NaCl were 1%, 5%, and 10%, respectively. Similarly, the high mitigation efficiency of calcium chloride ($CaCl_2$) on the acrylamide formation in fried potato crisps was also demonstrated.[246] Subsequently, the effect of mono- or divalent cations was systematically investigated. Added divalent cations, such as Ca^{2+}, were found to prevent acrylamide formation completely, whereas monovalent cations, such as Na^+, almost halve the acrylamide formed in the model system. Dipping potatoes into $CaCl_2$ solution inhibits the formation of acrylamide by up to 95% during frying. The sensory quality of fried potato strips, in terms of golden yellow color and crispy texture, is not adversely affected by this treatment.[247]

4.3.5. Addition of Antioxidants

The relationship between the antioxidant effects and the mitigation of acrylamide deserves to be discussed. Previously, many correlative tests have been performed and both positive and negative effects on acrylamide reduction have been demonstrated by using different kinds of antioxidants. Tareke[248] found that the addition of antioxidants (BHT, sesamol, and Vitamin E) to meat prior to heating enhances the formation of acrylamide, probably by protection of acrylamide against free radical initiated reactions. Meanwhile, decreased acrylamide formation has been found when adding rosemary extracts to the oil used for frying potato slices.[249] Furthermore, relatively lower amounts of acrylamide after the addition of a flavonoids' mix have also been reported.[250] A liquid flavonoids' mix was added to potato slices before frying, and a powder mix was also added to the potato slices after frying. After a 4 day incubation time, the acrylamide levels were detected to be reduced by up to 50% in the flavonoids' mix treatment.[251] Biedermann et al.[57] found a relatively weak reduction of the acrylamide formation by the addition of ascorbic acid to a potato model. Moreover, similar results were obtained by Levine and Smith[243] when using ascorbate as the additive.

The above observations indicated three relatives, the mitigation of acrylamide, the antioxidant capacity of antioxidants, and the antioxidant activity of model systems or food matrices. The relationship between each other sounds important and needs to be clarified. Summa et al.[211] found a direct correlation between acrylamide levels and the antioxidant activity of model cookies. Meanwhile, Ciesarová et al.[252] demonstrated that the effect of the spice antioxidants on the reduction of acrylamide contents is in close correlation with the antioxidant capacities of the applied spices in a model potato matrix. Under the guide of the close relationship between the mitigation of acrylamide and antioxidant activity, some new antioxidants were recently found to effectively reduce the acrylamide levels in various matrices. The virgin olive oil (VOO) having the highest concentration of ortho-diphenolic compounds is able to efficiently inhibit acrylamide formation in crisps from mild to moderate frying conditions.[253] The antioxidant of bamboo leaves (AOB) having the characteristic compounds of flavonoids, phenolic acids, and lactones was recently approved to effectively mitigate the potential risk of acrylamide in potato-based foods, fried chicken wings, and oriental fried bread sticks.[254] To elucidate the mitigation mechanism of antioxidants, Zhang et al.[255] investigated the effect of AOB and the extract of green tea (EGT) on the kinetics of acrylamide in the asparagine−glucose model system under microwave and low moisture heating conditions. Both studies indicated that addition of AOB and EGT could significantly reduce the formation rate constant (k_F) of acrylamide but could not significantly affect the elimination rate constant (k_E) of acrylamide. Such observation further clarified that addition of AOB/EGT could reduce the formation of acrylamide during the formation−predominant kinetic stage but could not reduce the acrylamide content during the elimination−predominant kinetic stage of acrylamide. Another possible explanation for the mitigation effect of antioxidants on the formation of acrylamide was related to the degradation and polymerization of acrylamide. A modest effect of the peroxides in oxidized oil on acrylamide elimination was observed, pointing toward an oxidative induced polymerization of acrylamide. The impact of oxidative processes on acrylamide elimination should not be neglected, since oxidized vegetable oil seems to promote degradation of acrylamide.[256]

With the development of the studies on the mitigation mechanism of acrylamide via the addition of antioxidants, key functional groups which play an important role in the mitigation process in these antioxidant compounds need to be clarified. Quantitative structure−activity relationship (QSAR) is a powerful method for the design of bioactive compounds and the prediction of corresponding activities that correlate with physical and chemical properties.[257] Previous studies used QSAR models to analyze and predict the antioxidant properties of a sequence of compounds, such as flavonoids.[258] Based on a QSAR study, researchers indicated that the antioxidant capacity is highly related to the number and location of aromatic hydroxyl groups of flavonoids.[259] Other researchers demonstrated the above finding via establishing the QSAR models of Trolox equivalent antioxidant capacity (TEAC).[260] Thus, the combination between the QSAR method and the mitigation study on the formation of acrylamide will be an interesting highlight for further investigation.

Besides the description of the above research progress, the irradiation and genetic modification techniques such as effective mitigation recipes are gradually acknowledged.[261] However, the optimization and mechanism of these recipes still need to be further studied. The main points of mitigation studies selected recently are highlighted in Table 4. Many additives used in various food matrices can be confirmed as

Table 4. Some Recent Studies and Findings on Mitigation of Acrylamide in Various Food Matrices

food matrix	mitigation recipe	main findings	ref
french fries	lactic acid fermentation for 45 and 120 min; blanching	79% and 94% of reduction, respectively	Baardseth et al.[234]
model systems	addition of glutamine, glycine, lysine, and alanine	76%, 70%, 57%, and 14% of reduction, respectively	Claeys et al.[219]
potato-based frying foods	selection of potato tubers	establishment of selection criteria by considering 16 parameters	De Wilde et al.[262]
french fries	microwave precooking	36%−60% of reduction	Erdŏgdu et al.[233]
water	ionizing radiation	significant degradation of acrylamide with 1.5 kGy of irradiation	Fan and Mastovska[263]
potato croquettes	coating with egg/breadcrumbs	80% of reduction	Fiselier et al.[236]
bread	long time fermentation	87% and 77% of reduction in whole grain and rye bran bread, respectively	Fredriksson et al.[264]
potatoes	controlled atmosphere storage and low-dose irradiation	effective decrease of reducing sugars in original materials	Gökmen et al.[261a]
asparagine−fructose model system	addition of divalent cations (e.g., Ca^{2+})	95% of maximal reduction	Gökmen and Şenyuva[247]
most relevant foods	legal measures	summarization of five proposed measures	Grob[265]
baked, fried and roasted products	addition of asparaginase	significant reduction of acrylamide	Hendriksen et al.[235]
fried potato model system	water treatment prior to frying, and removing some of the residual heat	inhibitory effect to a different extent	Ishihara et al.[266]
potato crisps	soaking in acetic acid solution	60% of maximal reduction	Kita et al.[238]
asparagine−glucose model system	addition of 1%, 5%, and 10% of NaCl	32%, 36%, and 40% of reduction, respectively	Kolek et al.[245]
potato model system	addition of citric acid and glycine	Citric acid limits the generation of volatiles, particularly the alkylpyrazines. Glycine increases the total volatile yield by promoting the formation of certain alkylpyrazines.	Low et al.[237a]
wheat and potatoes	agronomic and genetic approaches	reducing acrylamide precursors	Muttucumaru et al.[267]
model system	addition of trehalose or neotrehalose	76% and 75% of reduction, respectively	Oku et al.[268]
fried potato crisps	addition of $NaHSO_3$, $CaCl_2$, and L-cysteine	$CaCl_2$ is a potential agent in decreasing acrylamide formation in fried potato crisps and can be applied on an industrial scale	Ou et al.[246]
french fries	immersion with water or citric acid, and blanching	inhibitory effect to a different extent	Pedreschi et al.[231b]
bakery products	removing ammonium-based raising agents, long yeast fermentations, fortification of flour with $CaCO_3$, and lowering the dough pH	summarizing the effectiveness of mitigation methods	Sadd et al.[244]
potato crisps and french fries	addition of AOB	74.1% and 76.1% of maximal reduction in potato crisps and French fries, respectively	Zhang et al.[254a]
fried chicken wings	addition of AOB	59.0% of maximal reduction	Zhang et al.[254b]
fried bread sticks	addition of AOB or EGT	82.9% and 72.5% of maximal reduction, respectively	Zhang et al.[254c]

effective recipes for the mitigation of acrylamide. Their optimal addition levels also fall in the scope of permissible use levels and supply useful recommendations for industrial food processing.

4.3.6. Mitigation of Acrylamide and Sensory Attributes of Products

The use of mitigation recipes during food processing also requires the guarantee of sensory attributes. Current recipes, especially the pH modification, may also have an impact on the sensory product quality because low pH inhibits the Maillard reaction and contributes to the generation of undesirable flavors.[173] Acidification processing may result in a sour product taste.[238,269] However, this effect depends upon the soaking or blanching treatment, and the type and concentration of the acid applied. Addition of sulfur containing amino acids may also generate unpleasant off-flavors upon heating, which should be taken into account.[219] The use of $CaCl_2$ may improve product texture, but oppositely can cause a bitter aftertaste.[270] To cope with the impact on the sensory attributes, a compromise is needed between the mitigation effect of acrylamide and the sensory impact. To date, some investigations have been published specifically concerning this compromise. Anese et al.[271] found lactic acid fermentation in the presence of glycine leads to the most effective decrease in acrylamide formation (up to 70%) and simultaneously keeps original browning, flavor, sourness, and crispness of the deep-fried potatoes. Zhang et al.[254] found optimal addition levels of AOB based on the consideration of both remarkable mitigation effect and sensory attributes in potato-based foods, Western-style fried foods, and Chinese traditional fried foods, respectively. A quantitative descriptive analysis can be used for the sensory evaluation according to ISO 6658.[272] The descriptors may contain browning, sourness, flavor, texture, and crispness.

4.4. "CIAA" Toolbox

The CIAA "Toolbox" reflects the results of more than three years of industry cooperation to understand acrylamide formation and potential mitigation steps. Its aim is to provide brief descriptions of the mitigation steps evaluated and, in many cases, already implemented by food manufacturers and other partners in the food chain.[273] This approach allows individual manufacturers, including also small and medium size enterprises with limited research and development resources, to assess and evaluate which of the mitigation steps

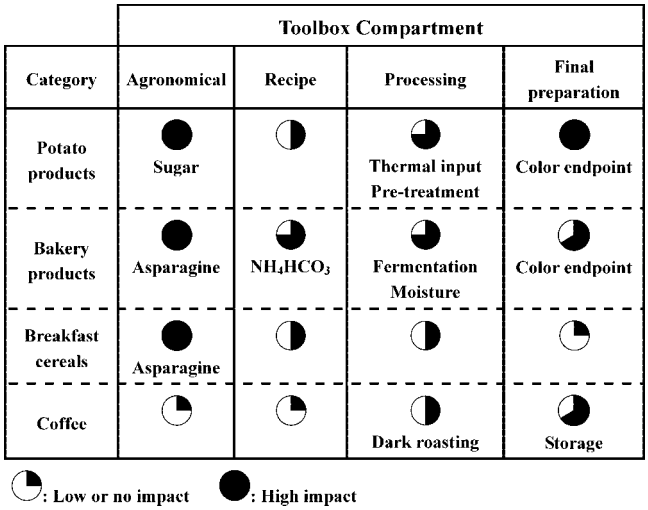

Figure 4. Main parameters and their impacts on the reduction of acrylamide in several characteristic foods.

identified so far may be helpful to reduce acrylamide formation in their specific manufacturing processes and products.

For the definition of CIAA "Toolbox" parameters, a total of 13 additional parameters grouped within the four major "Toolbox" compartments have now been identified, which include the agronomical (sugars and asparagine), recipe (ammonium bicarbonate, pH, minor ingredients, dilution, and rework), processing (fermentation, thermal input, and pretreatment), and final preparation (color end point, texture/flavor, and storage/shelf life/consumer preparation). These parameters can be selectively applied by each food producer in line with their particular needs and product/process criteria. In addition, the stage at which the different studies have been conducted, i.e. laboratory, pilot, or in a factory setting (industrial), is aligned to the potential mitigation measures. This approach ensures that all pertinent tests and studies are captured independent of their (immediate) applicability. Figure 4 showed the main parameters and their impacts on the mitigation of acrylamide in several characteristic foods. The greater the ratio of the black part, the more important the indicated parameter.

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

5. Conclusions

This review summarizes and presents the state-of-the-art trends of the analytical chemistry, formation, and mitigation recipes of acrylamide in various food matrices in detail. Especially, the research progress on the rapid, easy-to-use, and newly validated analytical methods, formation mechanism, and kinetics, possible mitigation mechanism, and new or improved mitigation recipes studied during 2006−2008 are highlighted. For the analytical chemistry, besides the pretreatment and instrumental improvement of GC-MS and LC-MS/MS, the fast recommended pretreatment, UPLC-MS/MS analysis, HPLC-DAD, GC-ECD, TLC, and recent optimized genetic and ELISA techniques significantly accelerate the analytical speed and efficiency of acrylamide in various samples. Meanwhile, some newly developed methods including CZE, MEKCC, TOF-MS, near-infrared spectrometry, and computer vision-based image analysis broaden the method selection for the analysis of acrylamide. For the formation of acrylamide, the Strecker degradation and *N*-glycoside pathway during the Maillard reaction are further studied while the importance of decarboxylated Schiff base and decarboxylated Amadori product is highlighted on a mechanistic basis. Special focus is concerned about the formation and impact factors of acrylamide in potato-based products, bakery products, cereal, coffee, almonds, and olives. Both acknowledged and new impact factors are discussed in detail. Also, the first-order formation/elimination model, mechanistic model, and nonisothermal model are successfully applied to the kinetic study of acrylamide. Mitigation recipes of acrylamide, maleimide, succinimide, and niacinamide may be generated besides the formation of acrylamide during the asparagine pathway. Thus, acrylamide can be controlled via inducing the reaction toward the formation of these three products. Besides acknowledged recipes, the use of exogenous additives, such as citric acid, proteins and amino acids, hydrogencarbonates, mono- or divalent cations, and antioxidant, is an effective method for the mitigation of acrylamide. The CIAA toolbox is a robust medium for the categorization and summarization of formation and mitigation of acrylamide in various food products.

For the outlook of future research, an acknowledged fact that acrylamide is formed in the Maillard reaction has been adequately demonstrated. The Maillard reaction is a cascade of consecutive and parallel reaction steps, whose complexity has been illustrated in related publications.[274] With the development of various mitigation recipes of acrylamide, the reduction mechanism needs to be further demonstrated. First, a mechanistic study is needed to clarify the key functional groups of the additives which play an important role in the mitigation process. Second, further study is needed to demonstrate the action positions of these functional groups on the reactions in the asparagine pathway. The preferable approach is investigating the formation variation of intermediates, such as Schiff base in the reaction between asparagine and fructose, the transform action from glucose to fructose via isomerization, and the formation of acrylamide from intermediates on a kinetic basis. Meanwhile, further study should optimize the compromise recipe between the high reduction effect and reasonable sensory attributes. Nevertheless, the edible safety of processing products after addition of mitigation agents needs to be simultaneously concerned.

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