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Review

Acrylamide in Food: Bibliometric Analysis, Chemical-Biological Mitigation, and Future Research Directions

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ABSTRACT: Due to the great global economic importance of coffee, the international market has increasingly focused on the safety and quality of coffee compounds, such as acrylamide, which forms during the roasting process. The importance of acrylamide lies in its health risks, including its carcinogenic potential. Various strategies have been developed to mitigate the acrylamide in foods, including optimizing roasting time and temperature and the use of strains to metabolize the acrylamide by using asparaginase (preacrylamide precursor) or acrylamidase enzyme (post acrylamide formation). While most of these methodologies have been extensively studied, limited information exists on the acrylamidase used to mitigate acrylamide in food. Understanding current acrylamide limits and related factors is essential for the effective management of coffee processing and processing procedures. This study aims to compile the current acrylamide limits for different foods (including coffee) and to present strains with potential acrylamidase production.

KEYWORDS: *coffee, acrylamidase, scientometric, bibliometric, baking products, acrylamide mitigation*

1. INTRODUCTION

The discovery of potential carcinogenic compounds like acrylamide (AA) and other furfurals, which have proven negative health effects, in a large portion of foods subjected to cooking or roasting processes has raised much concern worldwide. This concern is particularly due to their formation through complex reactions during the cooking and roasting process. The production mechanisms of these compounds are related to high temperatures, chemical molecules, and reducing sugars.[1]

This generation of undesirable products during thermal treatments is known as thermal processing contaminants, with AA being the most important. It is formed by the degradation of free asparagine in the presence of sugars.[2] The formation of AA in foods during cooking or frying occurs mainly as a result of the reaction between amino acids, such as asparagine, and reducing sugars under high-temperature conditions. Furthermore, the degradation of vegetable oils, especially polyunsaturated fatty acids,[1] can generate acrolein, which is oxidized to acrylic acid, a precursor of AA. Strategies such as pretreating potatoes in acetic acid solution before frying have been effective in significantly reducing AA formation due to the release of amino acids and reducing sugars during treatment.[3]

The International Agency for Research on Cancer (IARC) classifies AA as probably carcinogenic to humans, as there is limited evidence of carcinogenicity in humans, but presents sufficient evidence showing that the substance promotes cancer in experimental animals studies.[4] Moreover, AA has been characterized as a genotoxic, neurotoxic, and reproductive toxin.[5]

Several strategies have been investigated to mitigate AA in foods, including the use of salts, amino acids, irradiation, vacuum treatment, supercritical CO_2 extraction, high hydrostatic pressure, and a pulsed electric field, among others. Additionally, biotechnological approaches using microorganisms or cell-free extract/enzymes like asparaginases and acrylamidases have also been explored.[6]

Asparaginases are enzymes that are generally abundant in starch-based foods. They play an essential role in enzymatic hydrolysis by converting the precursor asparagine into aspartic acid, a compound that deactivates the AA production pathway, thereby controlling the levels of AA generated.[7] On the other hand, acrylamidases or amidohydrolases are enzymes that catalyze the decomposition of carboxylic amides into carboxylic acids and ammonia.[8,9] These enzymes are found in various natural environments[8] and play a crucial role in the transformation and recycling of nitrogen compounds in the environmental, contributing to the nitrogen cycle.[10]

Bibliometrics is a scientometric quantitative research method used to study the scientific literature. It is essential in review studies as it is a systematic approach, ensuring the quality of information and providing evidence from the scientific literature. This method helps understand the history, evolution, and current state of the art, identify patterns and

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trends, and provide insights to pinpoint research gaps, thereby aiding in decision-making for future studies.[11,12] An additional advantage of bibliometrics is its ability to offer a comprehensive overview of a specific topic and identify emerging concerns.[13] It also enables the creation of a distinctive viewpoint through sufficiently in-depth analysis.[14] There is a lack of studies regarding scientometric techniques of AA mitigation on foods.

Since coffee has a significant commercial strength worldwide, it could be considered a relevant source of exposure for AA. In this sense, there is a gap to be filled with effective solutions to adequately reduce AA for industrial-scale implementation, keeping the sensory qualities of coffee products. This study aims to conduct a bibliometric analysis, identify strains with potential for acrylamidase production, compile the current acrylamide limits for various foods (including coffee), and suggest possible future research directions.

2. BIBLIOMETRIC ASSAY FOR HISTORY, CURRENT STATE, AND TRENDS IN ACRYLAMIDE IN FOODS

2.1. Methods. The Web of Science (WoS) database was utilized to collect relevant scientific articles. The query, based on details provided in Figure 1, was conducted on 20

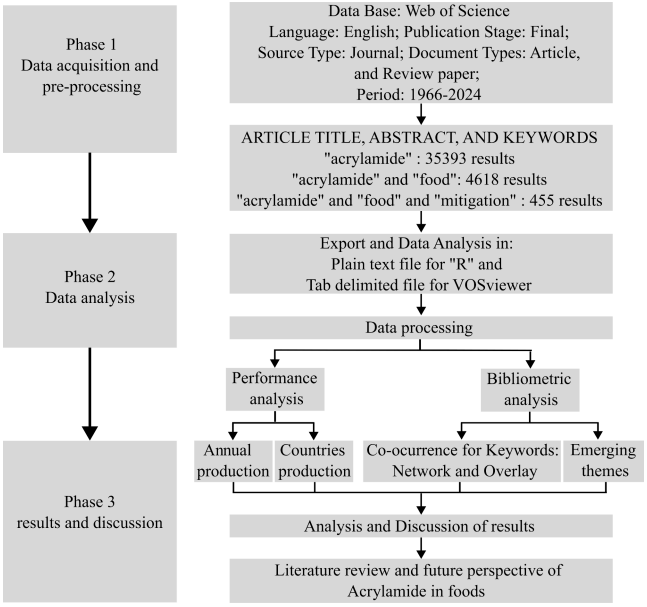

Figure 1. Methodology steps for the bibliometric analysis.

September 20, 2024. In the first phase, the keywords (1. "acrylamide"; 2. "acrylamide" AND "food"; and 3. "acrylamide" AND "food" AND "mitigation") were searched in titles, abstracts, keywords, and keyword plus. Only articles and review papers from the period 1966−2024 were considered.

In the second phase, the bibliographic data for the selected articles were exported as "Plain text files" and "Tab-delimited files" for use in "R" and VOSviewer, respectively. The data were then processed to analyze annual production, country-wise production, keyword co-occurrence expressed as an overlay network, and emerging themes. The third phase focused on interpreting the results and discussing findings related to acrylamide in food and its mitigation.

2.2. Results and Discussion of Bibliometrics. The initial query for "acrylamide" yielded 35,393 published documents, restricted to English language articles and reviews (Figure 1). The first document was published in 1966 (Figure 2a). The query was then refined using the keywords

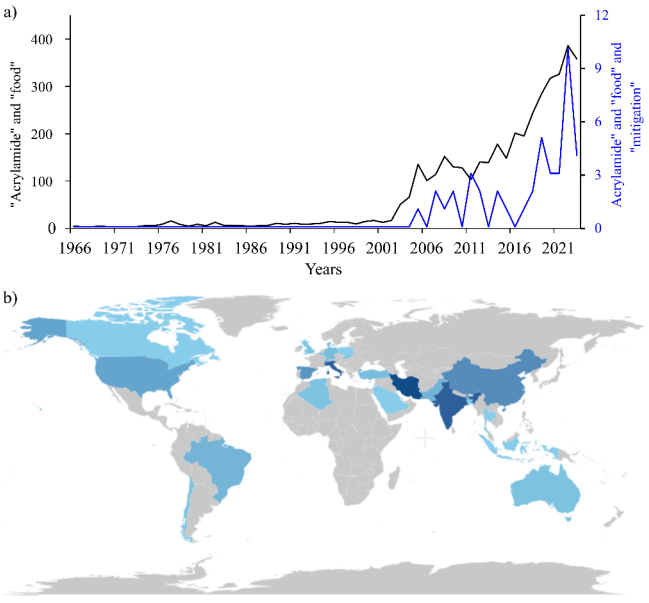

Figure 2. Annual scientific production (a) and country publication map (b).

"acrylamide" AND "food", resulting in 4,618 documents. Finally, the keywords "acrylamide" AND "food" AND "mitigation" were used, resulting in 455 documents.

For the results corresponding to the "acrylamide" AND "food" keywords, although the first scientific publication on AA appeared in 1966, there was limited interest from the scientific community until 2002. This is evident as the number of publications varied from 1 to 13 papers per year. After 2002, the number of publications increased linearly, reaching a peak of 381 publications in 2022. A similar trend was observed for the keywords "acrylamide" AND "food" AND "mitigation", with no publications on AA mitigation until 2004, followed by a linear increase over time.

Figure 2b highlights the countries addressing the issue of AA mitigation, with Iran, India, Italy, China, Spain, the USA, and Brazil being prominent in that order. Since Brazil is the major coffee producer,[15] its concern about AA mitigation is still a huge challenge due to the necessity to achieve the AA limits imposed by the governments of the countries that serve as the final destination for Brazilian coffee products, and well as other food products which contribute to the dietary exposure to AA.[16]

The overlay visualization from VOSviewer (Figure 3a) for the article keywords in the latest query (using "acrylamide" AND "food" AND "mitigation") illustrates the most co-occurring keywords over time. The colors blue, green, and yellow indicate the most used keywords in the oldest, intermediate, and newest years, respectively. These findings are generally consistent with the trend topic distribution by the bibliometric package of R (Figure 3b), which shows the elapsed time and the average year when the keyword was most used. Therefore, Figure 3, panels a and b are considered complementary. Overall, Figure 3 helps researchers analyze

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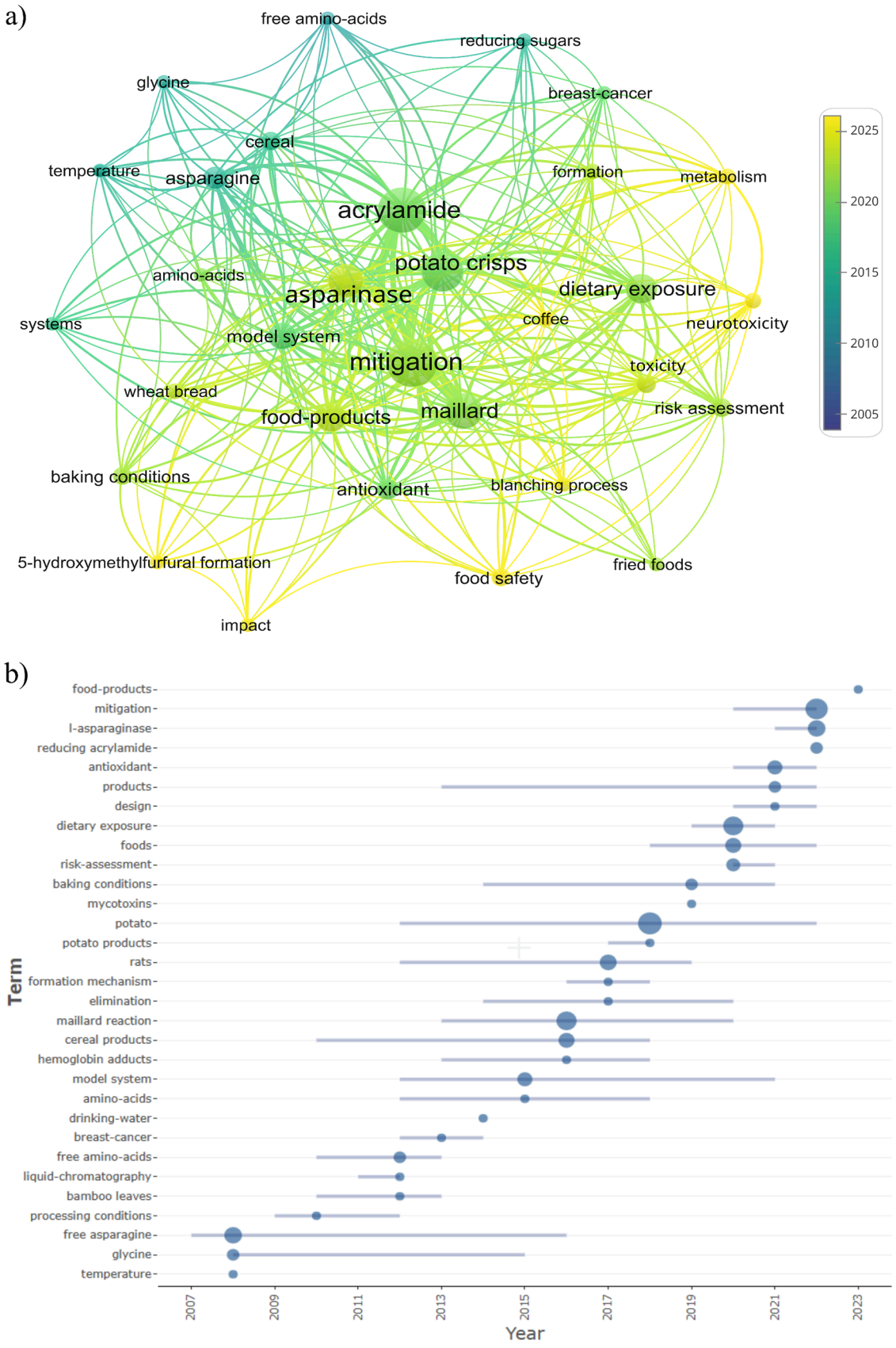

Figure 3. Overlay visualization (a) and trend topics distribution (b) of keywords on publication regarding query using "acrylamide" AND "food" AND "mitigation".

older and newer study interests, aligning their efforts with the most relevant and emerging research areas.[11,14]

Some of the earliest keywords (Figure 3a,b) such as asparagine, temperature, glycine, processing conditions, free

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amino acids, Mallard reaction, and others were more used in the years from 2007 to 2018. Conversely, terms like mitigation, reducing acrylamide, rats, asparaginase, risk assessment, food safety, metabolism, and neurotoxicity are the newest terms that point out the potential shift in the AA mitigation concerns and challenges around the world.

Additionally, these findings help researchers to detect potential gaps in the literature. For example, the absence of the word "acrylamidase" suggests an insufficient number of studies focusing on this mitigation option, despite its advantage of acting directly on AA molecules after the food production process.

3. ACRYLAMIDE

Acrylamide (AA) (Figure 4a) is a colorless, odorless, white crystalline aliphatic amide molecule, also known as 2-propenamide, acrylic amide, ethylene-carbamide, propenoic amide, vinyl amide, propenamide, and acrylamide monomer. In terms of its physical characteristics, it has a molecular weight of 71, a relatively high melting point of 84.5 °C, and a high boiling point of 136 °C at 3.3 KPa. AA is highly reactive and serves as a key compound in polyacrylamide formation. AA is highly polar soluble in water, alcohols, acetone, and acetonitrile, and slightly soluble in ethyl acetate and dichloromethane.[17] However, it is insoluble in hexane and other alkanes and alkenes. While it exhibits low but significant volatility, it does not show significant UV absorption above 220 nm and does not display fluorescence.[18]

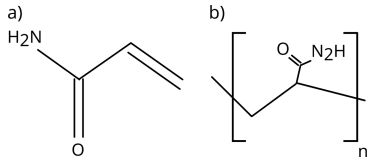

Figure 4. Acrylamide (a) and polyacrylamide (b) molecules.

AA is not present in fresh food, and it has been found in all food types prepared at high temperatures (>160 °C), including meat, bread, and potato products. Less amounts can be detected in cooked and microwaved foods. Even toasted tea leaves and roasted barley grains contain acrylamide in concentrations up to 570 and 320 ng/g, respectively. AA is generated in carbohydrate-rich foods such as frying, baking, roasting, and extrusion. However, it can form in low-moisture foods starting at 120 °C.[17] Both commercially processed foods and homemade meals tend to increase in AA with prolonged time and temperature of preparation. The surface color of products shows a significant correlation with AA levels in foods: the darker the surface, the higher the amount of acrylamide present.[18]

The acceptable levels of AA in food are outlined in EU Regulation 2017/2158. For example, the levels for roasted coffee and instant coffee are set at 400 and 850 μg/kg, respectively. These regulated levels are crucial, as research conducted in more than 20 countries has shown that European citizens consume AA daily at levels ranging from 140 to 1310 μg/kg of body weight, which are similar to levels observed in the USA. In adult and elderly populations (ages 20−79), coffee is a source of AA intake, accounting for 9 to 29% of total AA intake. This percentage increases to 38% to 60% for baked goods and snacks, depending on the country of origin.[19]

Regarding the formation of AA, the amide group can be protonated by medium to strong acids. Figure 5 presents the AA pathway for its formation/degradation. AA has reactive electrophilic double bonds and a reactive amide group, exhibiting weakly acidic and basic properties. AA has a very high technological potential; it can be industrially produced for the synthesis of polyacrylamide (Figure 4b), and it is frequently used in various production processes such as wastewater treatment, gel electrophoresis, paper manufacturing, ore processing, tertiary oil recovery, dye manufacturing, permanently pressed fabrics, and synthesis of other monomers. However, AA extensive production can leads to environmental contamination,[20] due to polyacrylamide degradation into acrylamide, it remains stable in water at elevated concentrations, and thus, it is of utmost importance to mitigate this residual acrylamide to reduce contamination.[21]

AA has been classified as neurotoxic, genotoxic, and teratogenic in animals, leading to intensified studies. Neurotoxicity has also been observed in humans. There is evidence regarding acrylamide formation in certain foods. Therefore,

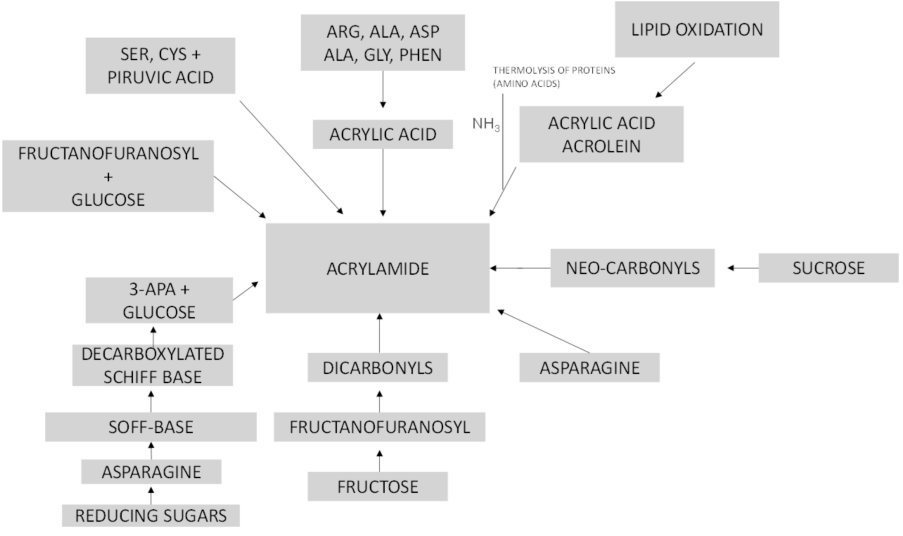

Figure 5. Pathways for acrylamide formation/degradation.

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efforts to mitigate acrylamide formation have been made by implementing various strategies aimed at minimizing or eliminating its precursors[22,23]

AA can be degraded by acrylamidases or amidohydrolases, enzymes that catalyze the cleavage of carboxylic amides into free carboxylic acids and ammonia.[9] These enzymes are widely distributed in nature and are involved in nitrogen metabolism. However, there is a lack of information about microorganisms capable of degrading AA for environmental removal. AA contains a vinyl group (CH_2═CH−) which binds to the thiol group from the microorganism's protein, causing toxicity.[24]

AA biodegradation has become increasingly important due to its negative impact on the environment. At the same time, there is vast potential and a huge scope for the development of research focused on more efficient AA degraders probable to minimize interference with sensorial attributes of food, especially when considering the application in an extremely sensorial beverage such as coffee. Thus, acrylamidase could play a crucial role in AA bioremediation.

3.1. Acrylamide Precursors. Figure 5 illustrates several metabolic pathways involved in the formation of acrylamide.

Alpha-dicarbonyl (α-DCs) plays a crucial role in the formation of harmful compounds through the Maillard reaction such as AA and advanced glycation end products. These are highly reactive, low molecular weight compounds,[25] commonly found in high-sugar or fat foods exposed to high temperatures. The Maillard reaction begins with the condensation of the reactive carbonyl of hexoses, like glucose and fructose, with the amino residue of amino acids/peptide/protein forming Schiff base and leading to the production of products through rearrangement and reversible isomerization.[26] The accumulation of α-DCs and their byproducts in the human body has been linked to the development of various chronic diseases. Therefore, it is essential to identify α-DCs in foods and understand the risks they pose.[27]

The α-DC also plays a crucial role in the chemical processes involved in caramelization reactions, different from Maillard reactions; caramelization promotes the formation of α-DC through lipid peroxidation and represents a relatively smaller proportion. It is generated in small quantities via the peroxidation of unsaturated fatty acids, where unstable primary oxidation products may decompose into it. α-DC.[28]

The free amino acid asparagine is likely one of the primary precursors of AA. Asparagine is a nonessential amino acid first isolated from asparagus juice in 1806,.[29] The addition of amino acids different from asparagine can decrease the amount of AA formed in thermal-treated foods. Among these, cysteine (Cys) is the most reactive, while other less reactive nucleophiles such as lysine (Lys), arginine (Arg), serine (Ser), and ascorbic acid produce similar condensation products.[30]

3.2. Acrylamide Toxicity. Polymeric AA is a harmless compound, but its monomer is neurotoxic to both humans and animals. In laboratory studies, AA has been shown to be carcinogenic in rodents and is considered probably carcinogenic for humans by the IARC. While AA has not demonstrated mutagenic properties in certain tests (organisms that do not have a true nucleus in their cells), long-term exposure has been linked to tumor formation in rats and mice. Acrylamide's neurotoxicity manifests as ataxia, distal skeletal muscle weakness, and numbness in the hands and feet. In the human body, acrylamide is oxidized to glycidamide (2,3-epoxypropionamide) through an enzymatic reaction, possibly involving cytochrome P450 2E1.[31,32]

As an unsaturated carbonyl compound with electrophilic properties, AA can react, via Michael addition, with biological nucleophilic groups including amines, carboxylates, aromatic and alkyl hydroxyls, imidazoles, and thiol groups of macromolecules (e.g., Cys residues), DNA, and proteins. This reactivity underpins its toxicity.[33]

AA and glycidamide can form hemoglobin adducts, although only glycidamide shows genotoxicity by forming adducts with DNA amino groups. Elevated AA levels can cause genetic mutations and cell transformation. Both can be detoxified in cells via conjugation with glutathione or hydrolysis. Smokers typically exhibit higher levels of AA hemoglobin adducts due to the presence of AA in tobacco smoke. Additionally, it is worth noting that dermal absorption of acrylamide is approximately 7% of oral absorption.[34]

Numerous studies, both in humans and animals, have been conducted to investigate the precise mechanism behind the toxicity of AA. Evidence suggests that AA toxicity primarily occurs due to oxidative stress.[35] *In vitro* studies have demonstrated that AA induces oxidative stress by forming Michael-type adducts with cysteine-containing glutathione (GSH), leading to a rise in reactive oxygen species production (ROS).[36] Moreover, through the action of CYP2E1, AA is metabolized into a more reactive metabolite, glycidamine (GA), which binds to GSH, leading to its depletion and further increasing ROS production, exacerbating oxidative stress.[37]

The testicular toxicity caused by AA is a public health concern as it has been shown to harm Sertoli and Leydig cells. Exposure to AA can induce direct genotoxic damage to DNA or interfere with reproductive processes, affecting hormonal balance. This endocrine disruption, including alterations in gonadal and pituitary hormones, is associated with histopathological changes and disturbances in spermatogenesis.[38] Additionally, dietary AA consumption has been associated with changes in sex hormones in women,[39] affecting oocyte quality and triggering oxidative stress, apoptosis, and epigenetic modifications.[40,41] These findings led the World Health Organization to classify AA as a reproductive toxin.

AA can stimulate gene expression and has been linked to neuroinflammation and neurotoxicity, contributing to the manifestation of depressive symptoms.[42] Consumption of AA is also associated with renal damage primarily caused by oxidative stress and toxicity. Such intoxication reduces antioxidant activities and increases lipid peroxidation, inducing inflammation, apoptosis, and DNA damage. AA-induced nephrotoxicity results in decreased urine volume and increased plasma levels of creatinine, urea, and blood urea nitrogen.[43]

Studies show that AA-induced cytotoxicity is closely associated with oxidative stress, resulting in cytotoxic and genotoxic effects. Efforts to mitigate the effects of AA have explored various plant bioactive constituents. Research has shown that certain plant bioactive constituents, such as black caraway (*Nigella sativa*),[44] pineapple (*Ananas comosus*),[45] purslane (*Portulaca oleracea* Linn.),[46] and quercetin can mitigate AA effects.[47] Additionally, many fruits, vegetables, leaves, seeds, and grains, such as capers, red onions, and kale, contain high resveratrol concentrations.[48] Dietary sources of resveratrol in food include the skin of grapes, blueberries, raspberries, mulberries, and peanuts. *Anthocleista nobilis* (G. Don.),[49] Curcumin[40] also have the potential to mitigate the adverse effects of AA, possibly through their antioxidant properties.[40]

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Moreover, studies reveal that AA exposure causes extensive hyaline degeneration, vascular congestion, irregularities in muscle fibers, and inflammation in cardiac muscle. These findings corroborate previous studies reporting AA-induced damage to cardiac tissue, including toxicity in cardiovascular development in zebrafish.[50]

3.3. Quantification Methodology for AA. Over the years, numerous analytical methods have been developed for detecting AA.[51−53] The sample preparation process typically involves extraction with water and solid-phase extraction for purification. AA can be analyzed using gas or liquid chromatography as well as capillary electrophoresis. Liquid chromatography coupled with mass spectrometry (LC-MS), in either single or tandem mode, allows for direct analysis of AA after derivatization. Additionally, UV detection also yields satisfactory results.[31]

Gas chromatography (GC) is also used to quantify AA in foods. In this method, the compounds of interest are vaporized conserving their structures in this way. AA and its metabolites were treated without the need for derivatization; in addition, the use of GC coupled to spectrometry (GC-MS) can isolate those molecules from their food matrix[54]

Depending on the matrix (food), AA can be extracted using water and/or organic solvents, but aqueous extraction is generally sufficient.[55,56] The FDA (Food and Drug Administration) advises one to avoid heating during AA extraction because this procedure could produce a large amount of fine particles that could saturate the solid-phase extraction columns used in later cleaning steps (FDA, 2003). When dealing with high fat samples, extraction with organic solvents is more efficient, with a mixture of water and acetone being recommended by various studies.[57] Another option is to include a defatting step before or in combination with the extraction process.[58,59]

Analytical methods for the identification and quantification of AA in foods are proposed worldwide. Some of them use LC-MS,[60] others with LC coupled with a tandem mass spectrometer, used to reduce sample preparation steps and, consequently, faster analysis, and still be able to work with a greater number of different matrices,[61,62] or LC with ultraviolet (UV) detection, although its use has been discouraged since that AA does not have a strong UV spectrum.[51] Yang et al. proposed an efficient method for AA derivatization using the thiol-olefin reaction with cysteine as the reagent combined with capacitive coupled contactless conductivity detection (C4D) for CE analysis. This method enables the analysis of labeled AA in just 2.0 min, with RSD values for migration time and peak area below 0.84% and 5.6%, respectively, demonstrating high precision and selectivity. Additionally, the C4D signal of the AA derivative shows a satisfactory linear relationship with AA concentration in the range.[63]

3.4. Food Safety. Most plant- and animal-derived foods undergo thermal processes such as high and low temperatures, sterilization, ultraviolet irradiation, and pasteurization to preserve their quality, extend their shelf life, and ensure food safety. However, in an investigation conducted by the FDA reported in 2021,[64] these processes can trigger the Millard reaction, formatting up to 126 potentially harmful substances. These α-dicarbonyl compounds are important intermediates and precursors of harmful products from the Maillard reaction, such as AA and advanced glycation end compounds, which exist in many thermally processed foods rich in sugar or fat.[65] These compounds impact food quality and serve as biomarkers for oxidative-stress-related diseases, including α-dialdehydes, α-diketones, and α-oxoaldehydes. They have been implicated in various chronic diseases, including diabetes and Alzheimer's. They play a significant role in protein glycation, a central process for Amadori product formation.[66] Therefore, the recognition and inhibition are crucial for food safety and quality and early disease monitoring.[27]

3.5. AA Enzymatic Mitigation Strategies. Preliminary procedures like blanching, saline immersion, storage temperature, and careful monitoring during heating, along with the addition of amino acids, antioxidants, and organic acids to lower pH, are effective in reducing aromatic amines. However, these methods can also impact sensory characteristics, including texture, flavor, color, and nutritional value of the final product. For instance, intense potato blanching can lead to mineral and vitamin loss, lower temperatures, and longer cooking times increase fat absorption, and substituting ammonium bicarbonate with sodium bicarbonate in cereal products reduces AA but increases sodium intake. Therefore, strategies to mitigate AA must balance food quality (nutritional, rheological, and sensory characteristics) and enzymatic approaches to decrease AA precursors or hydrolyze already formed AA.[67] Modern genetic techniques, such as chemical mutagenesis combined with genomics, offer promising reductions in AA formation.[68]

AA precursors, such as free asparagine and reducing sugars, are present in potatoes, coffee, and grains. The relevance of these agents varies among the species. While reducing sugars is predominant in cereal grains, the most crucial or limiting factor in AA formation in bakery products is the concentration of free asparagine. The asparagine content differs both between and within grains species. In common cereals, the average free asparagine content ranges from 426 ± 144 mg/kg to 1179 ± 359 mg/kg.[69]

The enzymatic hydrolysis of asparagine into aspartic acid and ammonia, facilitated by asparaginase (amidase), offers a promising strategy to mitigate heat-induced AA formation.[70] *In vivo*, deamidation acts as a molecular timer for biological events, including disease progression, and also forming significant proteins.[71] Factors like pH, buffer ions, ionic strength, and temperature influence the deamidation of asparagine residues in peptides and proteins.[72] These variables should also be tested for their effects on free asparagine in food products.[73]

The amidase (EC 3.5.1.4) is an intracellular enzyme that plays a vital role in carbon and nitrogen metabolism by breaking amide bonds to produce carboxylic acids. However, its catalysis process is hindered by disadvantages such as low stability and substrate specificity, which reduce its efficiency. Even though numerous approaches have been explored to obtain develop amidases with improved performance, the success rate is still inadequate.[74]

Enzymes that operate under moderate reaction conditions are highly specific and versatile biocatalysts. Nevertheless, their use in industrial processes is limited due to their low stability under harsh operational conditions and the challenges associated with their reuse and recovery, rendering them practically inaccessible.[75] Bacterial acrylamidase, in particular, deaminates AA to form acrylic acid and ammonia, and the acrylic acid further supporting bacterial growth.[10]

3.5.1. Asparaginases. Asparaginase (EC 3.5.1.1) is an enzyme found in animals, plants, and other organisms that is responsible for catalyzing the hydrolysis of asparagine into

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aspartic acid and ammonia. Aspartic acid plays a crucial role in the citric acid cycle and contributes to amino acid metabolism. In plants, asparagine is essential for energy efficiency and serves as the primary nitrogen storage form. Elevated levels of free asparagine are associated with increased AA formation, making asparagine a key enzyme in mitigation AA strategies.[76,77]

L-Asparaginase has a wide range of applications, particularly in the medicine and food industry. One of its important functions is to reduce the AA formation in foods during cooking at high temperatures, especially above 100 °C.[78] L-Asparaginase demonstrates potent biochemical activity within a pH range of 5 to 7, and at ∼37 °C. Although L-asparaginase is highly specific to asparagine, an amino acid, there exists a subgroup of enzymes called glutaminase-asparaginases, which are capable of processing both asparagine and glutamine. Glutamine, an amino acid, is structurally similar to asparagine.[67]

Crystallographic studies have revealed that both glutaminase-asparaginase and conventional L-asparaginase share a similar catalytic mechanism and structure, despite variations in substrate preference, pH sensitivity, and optimal operating temperature.[79] These insights are crucial for their application in the medical and food industry.[80]

In medicine, L-asparaginase is used to treat certain cancers, such as leukemia, by hydrolyzing asparagine and glutamine, thereby inhibiting malignant cell growth.[29] In the food industry, enzymes play a significant role in reducing AA formation during the cooking at high-temperature. For example, asparaginase can be applied to green coffee beans, to open their pores, ensuring effective contact between the enzyme and asparagine in the beans. Laboratory-scale experiments have shown a low dose (2000−6000 ASNU) applied to green coffee beans reduced AA concentrations by 55%−74%. These findings demonstrated the potential application of this enzyme in coffee processing.[81]

The treatment of coffee with asparaginase, combined with light-medium and medium roasting, has been shown to reduce the AA concentration by up to 39% in different Arabica coffee samples. However, when used at enzyme loads (up to 3000 ASNU), asparaginase may increase the acidity of the coffee.[16]

3.5.2. Acrylamidase. Acrylamidases are enzymes that facilitate the breakdown of carboxylic amides into free carboxylic acids and ammonia. Found abundantly in nature, these enzymes play an essential role in the nitrogen cycle.[82]

The flavor of coffee can be influenced positively or negatively by the type of enzymatic treatment process. Arabica coffee beans, known for their appreciated aroma in the final beverages, are severely affected, often leading to tasteless profiles.[83,84] Consequently, there is a current need to develop new methods for mitigating the presence of AA without generating undesirable secondary effects or negatively influencing the sensory quality of the coffee. In this context, bacterial acrylamidase presents a promising solution offering substantial potential for large-scale application to mitigate the presence of AA in coffee globally.[85]

The first studies on acrylamidases (also known as amidohydrolases or amidases) were conduced between the 1970s and 1980s, focusing on the similarity between acrylamidase and other hepatic enzymes. Those enzymes have demonstrated the capability to mitigate AA in levels in a variety of products, particularly foods.[86]

However, limited reports exist on microorganisms capable of effectively degrading AA from the environment. AA contains a vinyl group (CH_2═CH−) that reacts with the sulfhydryl group in microbial proteins, producing toxic effects. Acrylamidase production has been observed in only a few cultures, typically under stress conditions.[82,87] Some of these cultures are *Rhodococcus spp.*,[88] *Pseudomonas aeruginosa* BAC-6 isolated from industrial effluents,[89] free and immobilized cells of *Pseudomonas aeruginosa*,[90] *Moraxella osloensis* MSU11,[91] *Bacillus cereus*,[82] *Bacillus clausii*[92] and *Stenotrophomonas acidaminiphila* MSU12.[93]

The study of Bedade et al, 2017[10] identified and characterized a soil-isolate bacteria capable of degrading AA. It evaluated its optimal growth conditions and range of AA substrate concentrations that this isolate could degrade. The enzyme was analyzed for its optimal pH, temperature, substrate specificity, and activators and inhibitors such as metal ions and amino acids. This was the first report on the isolation of acrylamidase from *Arthrobacter* sp. DBV1 and its ability to degrade AA.[10]

According to Dev et. al., 2023[94] the acrylamidase from *Arthrobacter* sp. DBV1 exhibits insensitivity to the presence of amino acids. These findings suggest that this acrylamidase is a promising candidate for removal of AA already present in foods, demonstrating significant potential in a commercial context.

The sensitivity of acrylamidase to compounds that inhibit sulfhydryl proteins can also be evaluated. Heavy metals such as copper, lead, and mercury commonly inhibit amidases due to the presence of sulfhydryl groups in the enzyme's active site. Bedade and Singhal (2018) observed that the activity of acrylamidase was mildly inhibited by $CaCl_2$, $MgCl_2$, DMSO, $FeCl_3$, and ammonium persulfate.

3.5.3. Reducing AA Precursors on Raw Materials. Currently, research on the application of the acrylamidase enzyme to mitigate AA in various foods postformation remains limited after its formation. This gap presents numerous opportunities for further study, particularly in food matrices such as potato chips, French fries, coffee and soluble coffee, biscuits, snacks, bread, meat products, and fried snacks.

The reduction of AA precursors significant impacts the final AA content.[95] However, this effect depends on the relative initial levels of the precursors. When reducing sugars are present at levels higher than that of asparagine in food, reducing asparagine has a greater effect on minimizing AA formation. Several studies have demonstrated that AA formation is inversely correlated to sugar concentrations in potatoes, whereas in cereals such as rye and wheat, AA formation is primarily associated with asparagine content.[76] Additionally, the Maillard reaction involving chitosan, glucose, and asparagine along with the use of chitosans, has proven to be an efficient method for reducing AA levels.[96]

3.5.4. Acrylamidase-Producing Cultures. Given the current demands, there is an increasing need for studies exploring potential strategies to mitigate AA by using novel microbial cultures. Table 1 presents the same characteristics of cultures that produce acrylamidase enzymes.

Acrylamidase from *Cupriadivus oxalaticus* ICTDB921 demonstrated versatility and effectiveness in mitigating AA. This culture thrives in a medium with a pH range of 3 to 9 and temperature range of 30 to 80 °C. Additionally, it exhibits high resistance and robust growth across diverse pH and temperature conditions, making it a promising candidate for application in various matrices.[97]

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Table 1. Cultural Characteristics of Acrylamidase Producers

bacteria, pH, and temperature	characteristic	limitation	reference
Cupriadivus oxalaticus ICTDB921, pH 3–9, 30–80 °C	A highly versatile and effective molecule for controlling AA in diverse matrices. The bacterial culture demonstrated high resistance and robust growth across different pH and temperature conditions.		97
Stenotrophomonas acidaminiphila MSU12, pH 7, 32 °C	It is capable of degrading higher concentrations (30 mM) without any ecological effects.	It is a bacterial strain, is strictly aerobic, Gram-negative bacilli (rod-shaped), nonfermentative (glucose), and nonspore formers, with positive catalase and oxidase activity.	93
Geobacillus thermoglucosidasius, pH 2.5–8.5, 60 °C	It is capable of degrading more efficiently under neutral conditions and high temperatures.	The enzyme is most active at pH 5 to 7, with pH 6.0 providing the highest activity and above pH 7.2, and a temperature range of 60 to 70 °C.	98
Ralstonia eutropha, pH 2.5–8.2, 45–65 °C	Higher activity even at high concentrations of AA, up to 28 mM, under aerobic conditions at 30 °C, with pH 6.0.	Above pH 7.2 and 37 °C, the enzyme decreases to less than 50% of its maximum activity.	86
Moraxella osloensis MSU11, pH 7, 30 °C	The optimal growth temperature is 30 °C at pH 7.0. Additionally, the strain could assimilate glucose, sorbitol, mannitol, lactose, and sucrose with the purified degrading enzyme showing a specific activity of 52 U/mg of protein, with a final recovery of around 7%. It exhibits rapid degradation of AA and shows preferential growth in tropical conditions, indicating the potential of this bacterium for application in AA biodegradation and enzyme production.		91

Stenotrophomonas acidaminiphila, MSU12 is a strictly aerobic, Gram-negative rod-shaped, nonfermentative (glucose), nonspore-forming strain. It exhibits positive catalase and oxidase activity but tests negative for indole and urease activity. Optimal performance is achieved at 32 °C and pH 7.0, making it an effective acrylamidase with neutral pH ranges, although it has limitations in certain products. Under these conditions, it can degrade high AA concentrations (30 mM) without ecological effects.[93]

The *Geobacillus thermoglucosidasius* demonstrated optimal acrylamidase production in phosphate-citrate buffer (pH 2.5–6.5) and Tris-HCl buffer (pH 7.0–8.5). The enzyme functions best at pH values ranging from 3.2 to 9.2 for a temperature from 60 to 70 °C.[98]

Ralstonia eutropha retains its enzymatic activity at high concentrations of AA, (up to 28 mM), under aerobic conditions at 30 °C. The ideal pH for its activity is between 2.5 and 8.2, with pH peak activity at 6.0. Above pH 7.2, the enzyme activity decreases drastically. The pH dependence curve and ideal pH are similar to those of amidase from *Arthrobacter sp*. The optimal temperature for enzymatic activity is between 45 and 65 °Ct at 37 °C.[86]

Moraxella osloensis MSU11 is a Gram-negative *Diplobacillus* strain that is nonmotile, catalase-positive, and oxidase-positive and does not produce acid from carbohydrates. Optimal growth occurs at 30 °C at pH 7.0. The strain can assimilate glucose, sorbitol, mannitol, lactose, and sucrose. The purified degrading enzyme shows a specific of 52 U/mg of protein, with a final recovery of approximately 7%. It exhibits rapid AA degradation and exhibits preferential growth in tropical conditions, highlighting its potential for AA biodegradation and enzyme production.[91]

3.5.5. Bacteria that Metabolize AA. Another important and currently trending strategy for AA reduction involves the use of bacterial cultures that utilize AA as a carbon source for the cellular metabolism of these cultures. Table 2 highlights the characteristics of these bacterial cultures that produce acrylamidase enzymes.

Pseudomonas aeruginosa is a Gram-negative bacterial species recognized for its sensitivity and significant degradation capability when using immobilized cells for up to 48 h, after which activity decreases. The degradation process is inhibited by compounds, such as Cr^{6+}, Hg^{2+}, and Ni^{2+}. Compared to *Xanthomonas maltophilia*, *Pseudomonas aeruginosa* metabolizes AA more slowly under identical conditions, with maximal accumulations of acrylic acid and ammonia observed after 48 h. It effectively degrades AA across all tested concentrations (1–5%), with ammonia production detected after 48 h. The highest degradation is achieved at concentrations of 1–2%.[90,99]

Bacillus cereus exhibits optimal performance at temperatures between 25 and 30 °C and within a pH range of 6.8 to 7.0. Its growth is supported by a variety of carbon sources including glucose, fructose, lactose, maltose, mannitol, citric acid, and sucrose, with glucose being the most effective. *Bacillus cereus* responds to AA concentrations from 100 to 4000 mg/L demonstrating greater effectiveness at concentrations AA between 500 and 1500 mg/L.[82]

Enterobacter aerogenes effectively degrades AA at concentrations of 0.5% (w/v), within a pH range of 6.0 and 9.0, and at 25 °C. It also shows moderate degradation for other amides, including formamide, benzamide, acetamide, cyanoacetamide, and propionamide. These characteristics highlight the potential

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Table 2. Bacterial Characteristics of Acrylamidase Producers

bacteria (pH and T °C)	characteristic	limitation	reference
Pseudomonas aeruginosa	When compared to *Xanthomonas maltophilia*, it metabolizes AA slowly under identical conditions, with maximal accumulations of acrylic acid and ammonia observed after 48 h. *P. aeruginosa* degrades AA at all tested concentrations (between 1—5%), with ammonia detected after 48 h, although it is more efficiently degraded at concentrations of 1−2%.	Compounds such as Cr^{6+}, Hg^{2+}, and Ni^{2+} inhibit this degradation process.	90, 99
Bacillus cereus, pH 6.8−7.0, 25−30 °C	Growth is sustained by glucose, fructose, lactose, maltose, mannitol, citric acid, and sucrose, with glucose being the best carbon source. It reacts to different concentrations of AA ranging from 100 to 4000 mg/L incorporated but exhibits greater effectiveness at concentrations of AA between 500 and 1500 mg/L		82
Enterobacter aerogenes, pH 6−9, 25 °C	This strain degrades AA well at 0.5% (w/v), with a pH between 6.0 and 9.0 and at 25 °C. Moderate degradation occurs with other amides, including from amide, benzamide, acetamide, cyanoacetamide, and propionamide. These results highlight the potential of this bacterium in the cleanup of AA/amides in the environment.		100

of this bacterium in the environmental cleanup of AA/amides.[100]

3.6. Main Affected Foods. *3.6.1. French Fries.* Studies suggest that genetic modifying potato varieties to reduce or eliminate the reducing sugars and free asparagine amino acid before processing may be a practical strategy.[101] Furthermore, the amount of asparagine in potatoes is influenced by the sulfur content in the agricultural soil. Lower sulfur leads to lower asparagine content and consequently less AA formation after processing.[3]

3.6.2. Bakery Products. According to the Brazilian Association of Bakery and Confectionery Industry (Abip), there are over 70,000 bakeries in Brazil. In 2021, the bakery and confectionery market generated revenue of R$ 105.85 billion, reflecting a growth of 15.3% compared to 2020, as reported by the Association's president.[102]

The baking process is a significant contributor to AA production due to the Maillard reaction. This reaction greatly impacts the chemical, physical, and sensory properties of the final product while also promoting the development of bioactive and antioxidant compounds. Higher levels of AA are commonly detected in foods subjected to thermal processes such as frying and browning potatoes, cocoa beans, coffee roasting, and the baking of cereals and cakes.[103]

3.6.3. Coffee. Coffee is one of the most popular beverages worldwide, largely due to its pleasant aroma, which arises from the diverse volatile compounds produced during roasting. The International Coffee Organization (ICO) estimated that global coffee consumption increased by 4.2% to 175.6 million 60 kg bags in 2021/22, representing €165 billion annually.[104]

Arabica coffee (*Coffea arabica* L.) and Robusta coffee (*Coffea canephora*) are two important varieties in the coffee industry. Arabica is valued for its aromatic qualities, sweet flavor, and variety of tastes, whereas Robusta is associated with less desirable sensory characteristics, such as bitterness due to its high caffeine content and lower sweetness and acidity. These sensory differences, alongside with the production costs associated with each variety, contribute to the superior value of Arabica coffee beans.[105] This initial differentiation of products was based on caffeine content (Arabica vs Robusta), reflecting interspecific difference within the *Coffea* genus secondary differences, such as pre- and postharvest treatments (fermentation and roasting), further driving market differentiation. Arabica coffee is often treated as a specialty coffee owing to its higher presence of volatile compounds. However, increasing the productivity of Arabic coffee productivity has become increasingly challenging in recent years.[106]

Roasting is a traditional method of thermal treatment to achieve the desired flavor, dark coloration, and a brittle, porous texture in the beans, ideal for subsequent grinding and preparation. However, the high temperatures involved in roasting trigger a series of chemical reactions, dehydration, and significant changes in the microscopic structure. However, this process can also result in the formation of undesirable compounds, such as AA and furans.[19]

Sucrose is the main precursor of furans and methylfurans, while asparagine is the main precursor of AA. These findings shed light on the mechanisms behind the formation of these undesirable compounds in coffee.[107]

Unlike annual crops, such as potatoes and cereals, coffee is a tropical perennial crop that does not require annual sowing or planting. Annual crops can be easily manipulated to reduce the formation of AA precursors by altering varieties or changing

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production locations. However, this flexibility is not applicable to perennial crops like coffee. The quality of coffee beans and the final product is determined by factors such as soil composition, temperature, altitude, and water availability. Climate change, particularly rising temperatures, poses significant challenges to coffee production. Although strategies have been proposed to manage plantations, explore old species and varieties, and develop new hybrids to address climate effects, these efforts primarily focus on yield and flavor rather than AA precursors.[19]

Ground coffee can contain relatively high levels of AA, reaching up to 400 ng/g of powder. After brewing, no AA is detected in the coffee powder, suggesting that all of the AA transfers to the water. On the other hand, the AA thermal stability of AA has been extensibility reported. For example Strocchi et. al., 2022[19] found no significant decrease in AA levels in coffee, even after 5 h of heating. Table 3 presents the current AA limits.

3.6.4. Challenges to Mitigating AA in Coffee. During coffee roasting, complex reactions take place, leading to the formation of undesirable compounds, such as AA and other furfural compounds, which are considered harmful to health. These substances, categorized as thermal processing contaminants, are produced through a network of reactions during the thermal treatment of coffee beans. Among these, AA is the most significant contaminant, formed by the generated of free asparagine in the presence of sugars.[2] Interestingly, the same concentration of solids responsible for forming these harmful compounds also plays a critical role in developing the aroma and color of the final coffee products.

Table 3. Acrylamide Limits for Different Foods According to Regulation (EU) 2017/2158 of the Commission of 20 November 20, 2017[a]

food category	reference level (μg/kg)
French fries (ready to eat)	500
Packaged potato chips made from flesh potatoes and potato dough	750
Potato-based savory crackers	750
Other potato products made from potato dough	750
Fresh bread	
Wheat-based bread	50
Fresh bead excluding wheat-based bread	100
Breakfast cereals (excluding porridge)	
Bran-based and whole grain cereals, grains puffed by gun puffing process	300
Wheat and rye-based products	300
Corn, oat, spelt, barley, and lice-based products	150
Cookies and wafers	350
Savory crackers excluding potato-based crackers	400
Crispbread	350
Gingerbread	800
Products similar to other products in this category	300
Roasted coffee	400
Instant coffee (soluble)	850
Coffee substitutes	
a) Coffee substitutes exclusively from cereals	500
b) Coffee substitutes made from a mixture of cereals and chicory	500
c) Coffee substitutes exclusively from chicory	4000
Baby foods, cereal-based foods intended for infants and young children, excluding cookies and rusks	40
Cookies and rusks for infants and young children	150

[a]Non-whole grain cereals and/or not bran-based. The cereal present in the greatest quantity determines the category. The reference level applied to coffee substitutes made from a mixture of cereals and chicory considers the relative proportion of these ingredients in the final product. As defined in Regulation (EU) No 609/2013.

4. FINAL CONSIDERATIONS

This study successfully carried out a comprehensive bibliometric analysis, identified promising strains for acrylamidase production, and compiled the current acrylamide limits for various foods, including coffee. These findings provide a valuable foundation for understanding the current landscape of acrylamide research and mitigation strategies. Furthermore, the study highlights critical areas for future research, emphasizing the need for continued exploration of acrylamidase-producing strains and the development of innovative approaches to reduce acrylamide levels in food products. With possible advancements in technology and discoveries regarding sensory and physicochemical qualities, further studies are essential to maximizing microbiological and sensory quality results while mitigating the presence of acrylamide in foods. This will ensure that the final product achieves greater food safety for exportation or commercialization purposes, contributing to ongoing efforts to enhance food safety and public health.

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This work was conceptualized by DWCH and OF. The data acquisition and processing were performed by DWCH. Writing-original draft was made by JGS and DWCH. Writing-reviewing and editing was performed by LMFG, AFG, and OF.

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■ ABBREVIATIONS USED

AA	Acrylamide
Alpha dicarbonyl	α-DC
Arginine	Arg
Calcium chlorite	$CaCl_2$
Hydrochloric acid	HCl
Cysteine-containing glutathione	GSH
Deoxyribonucleic acid	DNA
Dimethyl sulfoxide	
DMSO	
	European Union
UE	
Ferrous chloride	$FeCl_3$
Food and Drug Administration	FDA
Gas chromatographic	GC
Gas Chromatography Coupled to Mass Spectrometry	GC-MS
Glycidamine	GA
High-performance liquid chromatography	HPLC
International Agency for Research on Cancer	IARC
International Agency for Research on Cancer	IARC
Lysine	Lys
Magnesium chloride	$MgCl_2$
Oxygen species production	ROS
Serine	Ser
United States of America	USA
Web of Science	WoS

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