

Chapter

Reduction of Acrylamide in Plant-Based Foods through Traditional and Innovative Fermentation Techniques

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Abstract

Acrylamide (AA), a heat-induced compound classified as a probable carcinogen, is commonly formed in plant-based baked goods and chips through the Maillard reaction. As the demand for healthier and more sustainable food production increases, innovative and natural solutions for acrylamide reduction have gained significant attention. This chapter explores the role of fermentation in reducing acrylamide levels, the impact of different starter cultures and fermentation parameters on acrylamide precursors, and the sensory quality improvements associated with this process. Furthermore, sensory analysis results are examined to assess consumer acceptance, providing valuable insights for industrial applications. By highlighting fermentation as a sustainable technology that enhances food safety and sensory attributes, this chapter aims to contribute to the scientific literature and support the development of safer, high-quality food products.

Keywords: fermentation, acrylamide reduction, plant-based foods, starter cultures, sensory analysis

1. Introduction

Plant-based food acrylamide reduction through fermentation research shows great promise because it simultaneously solves health-related problems and quality needs. Through fermentation processes, the formation of precursors that create acrylamide is lowered by affecting the transformation of both asparagine and reducing sugars. Through this process acrylamide reduction occurs while taste and texture of the food improves to meet consumer preferences.

Acrylamide reduction through fermentation occurs because asparaginase enzymes in microbial cultures transform the acrylamide precursor asparagine into safer breakdown compounds [1]. Earthen bacteria strains used in fermentation process influence chemical changes, which lead to different degrees of acrylamide decrease. The effectiveness of reducing acrylamide depends on the specific strains of bacteria, which researchers identify through studies [2].

Plant-based foods experience better acceptability due to fermentation because it enhances both their flavor and texture profile. People tend to pick fermented goods in sensory evaluations because these foods possess superior taste elements [3]. Fermented foods gain acceptance as reported by studies, so products with lowered acrylamide become more market desirable to consumers [4]. Fermentation emerges as a proper acrylamide reduction method, but traditional cooking creates considerable risks because heat and extended cooking durations increase acrylamide formation probabilities. A proper synergy between fermentation methods and defensive cooking procedures should be used to ensure both food quality and safety.

2. Fermentation and acrylamide

2.1 Acrylamide risk in plant-based foods

Acrylamide is a substance formed during the cooking of starchy foods such as potatoes, bread, or cereals at high temperatures, by frying, baking, or roasting. Because it appears in so many common foods that are cooked in this way, it has become an increasing concern for food safety and public health. Products such as biscuits, crackers, chips, coffee, and cereals, among others, may develop acrylamide as the result of high temperature cooking through baking, roasting, or frying or through processes that involve high temperatures of food, such as steam cooking, extrusion cooking, deep fat frying, or roasting. Raw food does not contain acrylamide, and the formation of acrylamide during thermal processing of carbohydrate-rich products is a direct consequence of thermal processing [5]. The majority of acrylamide is formed through the Maillard reaction, which involves a reaction between reducing sugars and the amino acid asparagine above 120°C [6, 7].

The process is accentuated at high temperatures and long heating times [8, 9]. Also, acrylamide is formed to the greatest extent when Maillard reaction occurs in dry heat cooking environments, as such heating increases in formation of acrylamide [6, 8]. From a compositional point of view, potatoes and other starchy foods containing high levels of free asparagine and reducing sugars are likely to be processed into more acrylamide [5, 7]. Research demonstrates that knowledge of raw material composition and processing conditions is essential for developing effective mitigation strategies. Acrylamide exists primarily in the following dietary sources:

1. The high starch content of potatoes in French fries and chips, along with their typical deep-frying preparation methods, make them a primary source of acrylamide [10].
2. The consumption of roasted coffee beans maintains elevated acrylamide levels [7].
3. Baked goods such as bread, biscuits, and breakfast cereals that undergo prolonged high-heat exposure [11, 12].
4. Toasted grains along with other wheat-based baked products appear frequently in daily food consumption habits [10].

The International Agency for Research on Cancer views acrylamide as a Group 2A probable human carcinogen since it likely causes cancer in humans [6, 11]. Studies

have found that mitigation strategies depend on knowledge about raw material composition as well as processing conditions. The major dietary sources of acrylamide are listed as being primarily:

- It is neurotoxic, and both cognitive and motor system impairments have been observed [11, 13];
- Nevertheless, there is a widely accepted background of carcinogenicity [14];
- Reproductive and developmental toxicity, especially under high exposure [15, 16];
- Its results consisted of hepatic and cardiovascular damage induced by the mechanisms of oxidative stress [15, 17].

Regulatory bodies assess the risk posed by acrylamide exposure through marginal exposure (MOE) assessments, which suggest that consumer risk exposure may have increased in recent years [7, 18]. Food regulatory organizations from different nations employ assessment results and standardized national criteria to determine their maximum allowed acrylamide levels. Research into acrylamide in plant-based products such as potatoes and cereals and coffee has received continuous regulatory attention from the EU, leading to benchmarks for these substances. European nations take the front position in implementing specific rules to lower the consumption of acrylamide while boosting food security monitoring systems.

According to Commission Regulation (EU) 2017/2158, food manufacturers operating in the European Union must follow mandated benchmark levels and reduction strategies for acrylamide substances in foods [10, 18]. The Codex Alimentarius Commission creates standardized testing standards and risk management procedures for public health through an international scope [9]. Food producers must always follow regulation-compliant mitigation procedures as outlined in official verifications while avoiding any changes to the approved methods [11, 12].

2.2 Relevance of fermentation strategies

The growing concern over acrylamide formation has motivated researchers to implement fermentation strategies as innovative food processing methods for acrylamide reduction. The production of fermented foods offers a natural and sustainable approach to enhancing the health profile of plant-based products, meeting clean-label requirements for both consumers and industry. The resistance of acrylamide to thermal processes is reduced due to chemical changes in food matrices induced by traditional or modern fermentation techniques involving lactic acid bacteria and yeast. The reduction of acrylamide during fermentation occurs through multiple operational pathways:

- Microbial metabolism plays a substantial role in depleting asparagine, the key amino acid precursor in acrylamide formation.
- Lowering the pH inhibits the Maillard reaction and, consequently, acrylamide synthesis.
- The profile of reducing sugars in food products can be modified to minimize the availability of Maillard reaction substrates [8, 11].

Tests show LAB fermentation reduces acrylamide levels in bread and vegetable-based substances, and yeast fermentation proves effective for coffee bean processing [19]. Noxferm technologies deliver a double result since they increase safety standards and create better flavor perceptions alongside longer shelf-life durations and nutritional value benefits.

The rise in customer interest for plant-based minimally processed items enables manufacturers to adopt fermentation methods that help fulfill regulatory needs and maintain product quality through EU Regulation 2017/2158. Product sustainability improves through fermentation because the technique reduces the dependence on chemical additives and significant formulation adjustments. Food safety benefits from fermentation because this process works as both a risk reduction measure and an enhancement technique, which integrates nutritional improvement with sensory quality development.

3. Fermentation techniques for acrylamide reduction

3.1 Traditional LAB fermentation

The fermentation process requires lactic acid bacteria (LAB) from grains and dairy products as well as vegetables to function. Production of lactic acid creates conditions that are hostile to microbes, leading to longer product shelf life together with superior product quality. Several studies now show that fermentation improves product safety along with nutritional quality [20].

LAB are gram-positive, non-sporing, non-respiring cocci or rod-shaped bacteria that are important microorganisms for food production, agriculture, and clinical applications. They ferment carbohydrates to produce lactic acid [21]. The four main genera that scientists agree on are *Streptococcus*, *Leuconostoc*, *Pediococcus*, and *Lactobacillus*. *Weissella*, *Enterococcus*, and *Aerococcus* are new genera added in recent reviews. These harmless bacteria produce organic acids and other metabolites through the hydrolysis of food sugars. LAB has a GRAS (Generally Recognized as Safe) status, enhanced by its widespread use and occurrence. There is no clinical evidence of adverse effects for these fermenting bacteria except for enterococci, as per the EFSA's "Panel on Biological Hazards" [22].

It has been shown that LAB, which are commonly used in conventional fermentation processes, reduce acrylamide formation through the degradation of asparagine and other reducing sugars. Research conducted on potato slices using LAB-fermented solutions showed a significant reduction in acrylamide formation when the potato slices were fried [23, 24]. LAB strains in sourdough fermentation, together with prolonged fermentation times, help decrease acrylamide formation in bread-making processes [25, 26].

The reduction of acrylamide in food production depends heavily on the fermentation processes conducted by lactic acid bacteria (LAB). Environmental conditions developed by microorganisms through enzymatic activity lead to reduced pH levels that help decrease acrylamide formation from asparagine precursors. The food industry relies on LAB as an essential approach to reduce acrylamide content within diverse food products [8].

3.2 Sourdough fermentation and LAB-yeast synergy

During sourdough fermentation yeast and LAB together create a synergistic reduction of acrylamide in bread alongside baked goods. The incorporation of LAB into

sourdough fermentation means lower acrylamide content as well as delivers better texture and taste to the product. Through this strategy, researchers succeeded in lowering acrylamide formation and created higher nutritional value within the bread product [19].

The blending of yeast with lactic acid bacteria (LAB) through sourdough fermentation produces successful acrylamide content reduction in food items. By employing this method, the chemical acrylamide formation decreases, and the products achieve a better texture together with tastier sensory properties. The fermentation process that combined sourdough with yeast resulted in substantial acrylamide reduction in whole-wheat bread compared to yeast fermentation alone [27].

Yeast and LAB fermented whole-wheat bread contained acrylamide levels between 6.9 and 20 µg/kg, whereas yeast-only bread contained 47.6 µg/kg [27]. Specific sourdough recipes reduced acrylamide formation by 79.6% in rye crispbread [28]. *Lactobacillus paracasei* generated the smallest amount of acrylamide (131.06 µg/kg) through its impact on dough acidity and glucose content [29].

Through its nutritional advantages and premium bread quality, the technology offers exciting commercial prospects especially when it integrates frozen products with substitutions of other ingredients [30]. Sourdough represents one of the oldest technologies through which people produce cereal-based foods. The traditional sourdough method allows fermentation by wild yeast and *Lactobacillus* bacteria found in raw materials instead of contemporary yeast (*Saccharomyces cerevisiae*) commercial methods [31].

The fermentation process used in sourdough bread production leads to productive acrylamide content reduction. Research shows that bread samples achieve reduced acrylamide content when fermentation proceeds for longer durations with increased sourdough concentrations up to 20% [25, 26].

3.3 Probiotic and functional microbial fermentation

Studies show that fermentation using *Lactobacillus* together with yeast produces promising results for lowering acrylamide concentrations. Adding probiotics to plant-based substrates such as soy and coffee beans promotes the breakdown of asparagine and other cooking precursors that lead to acrylamide formation [19]. The application of probiotic fermentation creates superior safety conditions while improving the nutritional benefits of final products.

The fermentation process with probiotics utilizes plant-based materials, such as fruits, vegetables, and cereals, to replace dairy substances in food production. Fermentation of soy drinks with sea buckthorn syrup and probiotic co-cultures of *Lactobacillus paracasei* and *Bifidobacterium animalis* enhances both bacterial viability and antioxidant activity [32]. Different microbial strains that are mixed during fermentation result in the generation of various functional attributes. A combination of *Saccharomyces cerevisiae* var. *bouardii* with kombucha microorganisms during fermentation results in probiotic mead, which shows better probiotic survival along with enhanced antioxidant features [33].

4. Mechanisms of acrylamide mitigation in fermentation

4.1 LAB mechanisms: pH, adsorption, precursors

Plant-based foods undergo acrylamide reduction during fermentation through various biochemical transformations. Acrylamide reduction occurs through enzymatic

asparagine degradation and specific microorganisms that enhance this process. The reduction of acrylamide occurs via the enzymatic degradation of asparagine, facilitated by specific microorganisms. Several mechanisms contribute to this reduction, which will be discussed in the following sections. Asparaginase breaks down asparagine into aspartic acid and ammonia through its enzymatic activity, thus blocking acrylamide formation when food undergoes high-temperature cooking [1, 34].

4.1.1 Reducing sugars

Fermentation by lactic acid bacteria (LAB) reduces acrylamide formation by controlling the amounts of reducing sugars and asparagine, both of which serve as key precursors in the Maillard reaction. The decrease in precursor amounts because of LAB strain enzymatic activities prevents thermal processing from producing detectable levels of acrylamide. LAB fermentation leads to decreased concentrations of glucose and fructose in fermented food products. Studies have shown that LAB treatment of fried potato products reduces the levels of essential reducing sugars that create conditions for acrylamide formation [24]. The impact of LAB strains on lowering asparagine concentration remains mild, thus only mildly reducing acrylamide formation potential.

The reduction in glucose and the simultaneous decrease of fructose along with asparagine affect Maillard reaction potential, thus lowering the final acrylamide content. LAB fermentation proves to be a powerful method for reducing acrylamide. The central role of reducing sugars in acrylamide formation can be modified through LAB fermentation, alongside alternative cooking techniques. Research suggests that adding Vitamin C and Vitamin B1 to food products reduces acrylamide concentrations. These vitamins interfere with the Maillard reaction; however, they do not affect LAB fermentation [35]. The cooking process determines how acrylamide is formed. Air frying results in reduced acrylamide content compared to traditional frying methods, as reported in [8], due to differences in heat transfer mechanisms and temperature profiles.

Acrylamide is formed through the Maillard reaction, which depends on reducing sugars that serve as key precursors in food processing. LAB fermentation has the potential to decrease acrylamide content by reducing both reducing sugars and asparagine in food substrates. The reduction of reducing sugars is the primary mechanism for minimizing acrylamide formation, while LAB fermentation has a lesser effect on amino acid modulation. Optimizing LAB fermentation techniques for different food products and processing conditions requires further research. A comprehensive framework for acrylamide reduction in foods can be developed by combining LAB fermentation with other mitigation strategies, including enzymatic treatments and modifications to cooking methods.

4.1.2 Asparagine depletion

The reduction of acrylamide depends on fermentation conditions, which include pH levels, temperature settings, and time duration. The effectiveness of acrylamide reduction depends on fermentation parameters within the pH range of 4.5–6.5 and temperatures between 32 and 42°C, which enhance microbial fermentation activities [36].

4.1.3 pH reduction and maillard inhibition

Acrylamide reduction is enhanced at lower pH values between 3.5 and 4.5 because acidic conditions inhibit the Maillard reaction [30, 36]. LAB grow best at

temperatures ranging from 30 to 37°C and effectively contribute to acrylamide reduction within this range [36, 37]. The process of acrylamide reduction through fermentation becomes more effective with longer fermentation periods extending between 12 and 24 hours [25].

LAB fermentation of Habanero peppers results in better flavor profiles because it adjusts pH levels and stimulates volatile compound synthesis. The pH value of Habanero peppers typically decreases from 4.78 to 4.47 when LAB fermentation occurs [38]. The production of organic acids becomes more favorable at lower pH values because these acids enhance both the preservation and flavor complexity of the peppers [39]. Food producers utilize LAB fermentation as a standard process because these microorganisms enhance product quality by changing pH values. The fermentation process generates lactic acid and various organic acids that decrease pH values while creating essential sensory features of fermented foods. LAB strains that speed up pH reduction in kimchi fermentation cause fast sugar consumption and acid generation. The optimal sensory preference for kimchi occurs when acetic acid and lactic acid coexist during fermentation because pH-based acid equilibrium controls flavor development [39, 40].

Volatile and non-volatile compound generation during LAB fermentation is directly influenced by changes in pH. LAB fermentation of cereal products produces ethanol, acetic acid, and carbon dioxide, which contribute to the texture and appearance of the final product. The reduction in pH leads to increased compound formation, enhancing the sensory qualities of the final product [41].

4.2 Chemical changes in fermented plant-based foods

Through lactic acid fermentation, the production of probiotic-rich foods becomes possible using microbes such as *Lactobacillus* and *Bifidobacterium*, among others. The preservation process enables these bacteria to convert sugars into lactic acid, which establishes a suitable environment for probiotic growth. Antioxidant components and bioactive properties significantly increase after fermenting probiotic purees containing different strains, including *Lactobacillus fermentum* and *Lactobacillus plantarum*. Complex laboratory studies have shown that specific microbial populations flourish under precisely engineered lactic acid fermentation conditions, developing nutritionally enhanced products that can greatly support public health when consumed daily with over 10 billion CFU per serving [42].

4.2.1 Role of amino acids

The development of acrylamide during LAB fermentation depends significantly on amino acids since these compounds interact through the Maillard reaction primarily with sugars. Several factors influence the concentration of acrylamide present in final products because different amino acids participate in the reaction. Asparagine functions as the leading substance that leads to acrylamide synthesis. Throughout fermentation, the treatment of asparagine with reducing sugars results in oxoaldehyde and oxoacid formation, which significantly enhances acrylamide production [43].

Acrylamide production during Maillard reactions occurs when valine, alanine, phenylalanine, and leucine amino acids participate in the process to generate materials that function as precursors for acrylamide synthesis [44]. The amino acid essential to the Maillard reaction to produce acrylamide is asparagine, which particularly reacts with reducing sugars to produce acrylamide. Cys, Lys, and Glu are involved in

Maillard reactions (flavor, etc.) and other biochemical changes during fermentation, but they are not the biggest contributors to acrylamide formation in amino acids [45].

Most other amino acids are generally less directly involved in acrylamide formation than asparagine. Various amino acids can participate in the Maillard reaction, but only asparagine is indispensable for the formation of acrylamide because its side-chain amide group is important for the unique reaction mechanism related with acrylamide formation [45]. As such, all other amino acids, even if engaged in Maillard reactions with effects on flavor and the formation of bioactive compounds, are not acting in the same manner as in product formation as during acrylamide formation during LAB fermentation or cooking.

However, some other studies suggest an indirect or modulating role of other amino acids in acrylamide formation. For instance, glutamine (Gln) could increase the amount of acrylamide production by the release of ammonia in a higher-temperature gradient, which potentially elevates the supply of the acrylamide precursor indirectly [36]. In thermal degradation, serine (Ser) can create substances that can be regarded as acrylamide precursors [46]. It is reported that certain amino acids such as alanine (Ala) and phenylalanine (Phe) can also affect the development of Maillard reactions, producing a variety of aroma compounds [47]. On the other hand, proline (Pro) and glycine (Gly) have been found to suppress the formation of acrylamide through the binding with reactive intermediates to minimize the exposure levels of acrylamide [47, 48].

Laboratory studies have confirmed that cysteine and lysine amino acids successfully decrease the amount of acrylamide formation during heating. The amino acids participate in competitive interactions with asparagine or modify the mechanisms of chemical changes that occur [49].

The ability of proline and glycine to reduce acrylamide formation increases notably when their amounts increase [44]. The formation of acrylamide becomes less challenging when glucose and similar reducing sugars are present during processing. Heat processing between amino acids and reducing sugars triggers chemical reactions that produce higher amounts of acrylamide [50]. Amino acids affect acrylamide formation by complex reaction processes that depend on the types of amino acids and fermentation parameters such as pH value and temperature and reducing sugar concentrations.

During fermentation the prevention of acrylamide formation occurs through non-toxic compound generation from acrylamide by cysteine and lysine chemicals. The reduction of acrylamide occurs at higher pH levels and temperatures because the reactivity of these amino acids intensifies under said conditions. Mechanism of action:

- Both cysteine and lysine contain reactive functional groups that initiate acrylamide reactions to produce stable non-toxic compounds. Acrylamide reacts with amino acids through first-order kinetics because of their direct structural relationship.
- Cysteine: The elimination rate of acrylamide through cysteine reaches up to 94.4% when applied. Acrylamide reactivity increases when pH levels are elevated, which leads to better acrylamide reduction during processing [51].
- Lysine: The reactivity of lysine remains high when its molar ratio with other substances increases. The compound demonstrates effectiveness for acrylamide

reduction and works best when combined with glucose during heat-based processing [50].

- Interaction with glucose: The addition of cysteine and lysine to glucose solution results in improved acrylamide elimination performance. This case illustrates the beneficial impact these amino acids have on blocking acrylamide generation throughout various food production methods.
- Impact of other amino acids: Cysteine and lysine decrease acrylamide levels, yet glutamine and possibly other amino acids may increase acrylamide formation. The opposite reaction patterns between these amino acids demonstrate the requirement for selecting appropriate ingredients when food processing to prevent acrylamide formation [49].

During LAB fermentation, amino acids either raise or decrease acrylamide levels through their chemical interactions. These amino acids show effectiveness in combination with pH conditions and temperature levels as well as reducing sugars, but controlling acrylamide formation proves challenging.

In summary, although asparagine is the dominant and essential precursor for acrylamide formation, the distribution and the reaction conditions also contribute to the level of acrylamide generation. This emphasizes the necessity to consider the complicated interactions between amino acids and processing parameters in strategies to reduce acrylamide.

4.2.2 Food matrix and fermentation conditions

Acrylamide production experiences significant chemical modifications in plant-based foods throughout fermentation. A decrease in acrylamide production in heated foods occurs because fermentation reduces the starting materials used for acrylamide formation. Antifungal food processing modifies both nutritional elements and acrylamide risk levels in plant-based food. The fermentation process and added substances in various plant-based meat products influence acrylamide content [52].

Besides microbial activity, other components in the food matrix, including dietary fiber content, protein-carbohydrate interactions, and water activity, play important roles concerning acrylamide formation. Higher matrix density could impair heat transfer and reduce the extent of Maillard reaction, leading to lower acrylamide content [53, 54].

The physical structure of the food (e.g., purée vs. solid slices) also influences acrylamide formation dynamics, as a denser matrix may restrict the diffusion of reactants like free asparagine and reducing sugars during heat treatment [55, 56]. Furthermore, matrix viscosity and moisture-binding ingredients such as hydrocolloids and dietary fiber could influence acrylamide formation by modifying water activity (a_w), an important factor in Maillard reactions [57, 58].

Enzymatic treatments (such as asparaginase) are more effective in matrices with moderate pH and low buffering capacity, conditions that allow better enzyme access and activity [56]. Plant matrices with high levels of polyphenols may also serve as scavengers of free radicals generated during processing at high temperatures, and thus, contribute to acrylamide chemistry [54].

Fermentation has been observed to lower the total sugar content of food products, which directly translates to lower acrylamide formation. The *Bacillus* strains used

in potato slice fermentation led to a 96.1% decrease in acrylamide content before deep-frying [23]. Long-term bread fermentation using sourdough resulted in reduced acrylamide formation, and specifically, using 20% sourdough starter maintained better gluten quality compared to higher concentrations [25]. The effectiveness of acrylamide reduction through fermentation depends on the specific fermentation methods used because different techniques produce dissimilar results. The results of acrylamide formation and food safety vary based on fermentation conditions, together with the selected microorganisms.

In addition, the matrix structure of plant foods, for example, the fat content and porosity, may favor or impair acrylamide formation during thermal treatment. For instance, in the high-fat matrix, the mobility of acrylamide precursors could increase, enhancing their reactivity [59]. On the contrary, high-fiber or viscous mediums may impede precursor migration and water evaporation, leading to decreases in the potential of acrylamide risks [56, 57].

Fermentation products are also modulated by the food matrix. Organic acids (e.g., lactic acid) generated during fermentation may bind or decompose acrylamide precursors, but the degree of their effects depends on the buffering capacity of the matrix and the mutualistic relationship with acidic molecule retention in the matrix [60]. In the case of meat analogs with soy, pea or a mycoprotein base, the incorporation of precursors in the protein network reduced acrylamide formation in the presence of reducing sugar [61]. Also, in cereal-based matrices, the effect of fermentation on gluten and arabinoxylans characteristics, affecting moisture holding/trapping and thermal conduction, which indirectly have an influence on acrylamide levels, has been observed as well [54].

5. Enzymatic approaches: The case of asparaginase

5.1 Enzyme mechanism and specificity

Asparaginase enzyme functions chips by transforming L-asparagine into its end products, aspartic acid and ammonia. Thermal processes halt the conversion process, preventing asparagine from participating in the Maillard reaction and subsequently reducing acrylamide formation. The enzyme demonstrates reactivity toward asparagine while it does not affect glutamine or other amino acids, thus helping maintain food matrix nutritional value and sensory quality [62, 63]. Research into food enzymatic treatment techniques has discovered L-asparaginase as an effective method to decrease acrylamide levels in starchy foods. L-asparaginase transforms L-asparagine (the acrylamide precursor) into L-aspartic acid, thus halting the acrylamide production process that occurs during high-temperature extended cooking of processed foods [5, 10]. Enzyme sources and applications:

- Multiple microbial strains of L-asparaginase, such as *Streptomyces koyangensis* SK4, *Fusarium culmorum*, and *Bacillus spp.*, have demonstrated acrylamide reduction in starchy food products [64, 65].
- When L-asparaginase from *Streptomyces koyangensis* SK4 was tested under optimal conditions, it reduced acrylamide content in potato chips by about 81%. In vitro enzyme activity reached its peak when potato chips were treated with sodium chloride treatment before undergoing air frying at 160°C. The authors

noted that enzyme concentration and other processing conditions need to be optimized for maximal reduction [65].

- The acrylamide levels in potato chips and sweet bread decreased by 94 and 86%, respectively, when using L-asparaginase derived from *Fusarium culmorum* (ASP-87). The authors stated that the enzyme also inhibited acrylamide levels in fried and baked starchy food products and could be used for commercial production [66].
- The tested enzyme from *Bacillus spp.* L-asparaginase demonstrated complete acrylamide reduction in potato slices after a 30-minute treatment. *Bacillus spp.* L-asparaginase I achieved better acrylamide reduction after purification and increased concentration during testing on potato slices [67].
- The results from L-asparaginase enzyme testing demonstrated that various enzyme concentration levels achieved better acrylamide reduction with enhanced potato chip sensory quality than the control group [68].

Asparaginase is selected as a prime example because it is the most selective toward L-asparagine, the main precursor of acrylamide in heat-treated food. Such specificity makes it possible to achieve a large decrease in acrylamide formation with no harm to the content in the remaining amino acids and the nutritional and sensorial properties of the food. The successful reduction of acrylamide by asparaginase has been confirmed in several strains of microorganisms and food products and serves as an excellent example of the use of enzymatic application relative to food safety improvement. Studies by Mottram et al. [46] and Zyzak et al. [69] proposed the Maillard reaction step that led to acrylamide formation in which asparagine was found to be the main precursor, and Amrein et al. [70] demonstrated that asparaginase use could be a practical means to decrease acrylamide in fried potato products. Therefore, asparaginase is a unique and prototype enzyme illustrating how enzymatic methods can be used to mitigate food contaminants [46, 69, 70].

5.2 Applications in fried, baked, and roasted products

Research indicates that the use of asparaginase throughout plant-based food production and fermentation activities effectively lowers acrylamide formations.

- *Potato-based products*: Conditions optimized for frying allowed the application of asparaginase before potato processing to decrease acrylamide formation by 81% [65].
- *Cereal-based baked goods*: Asparaginase treatment reduced the acrylamide content in baked cookies as well as breads and biscuits by 97 percent. Enzymatic pre-treatment produced an 89% reduction of acrylamide in fried pizza crusts, yet wood-fired pizza cuts displayed only a 50% acrylamide reduction [71, 72].
- *Coffee*: The enzyme treatment of green coffee beans produces an 80.7 and 75.8% acrylamide reduction in light roasts and dark roasts, respectively [62].
- *Fried dough products*: Model system research demonstrated that using asparaginase for dough treatment yielded about 90% reduction in acrylamide formation [73].

- The enzymatic system produced by *Aspergillus fumigatus* through Acrylamide amidohydrolase breaks down acrylamide and other substances in food products until reaching 95% degradation. This enzyme is used in food safety and quality management as it serves as an effective biocatalyst. After all, it functions as a vital instrument to convert acrylamide into harmless decomposition products through hydrolysis. Acrylamide amidohydrolase uses enzymatic action to break down acrylamide into acrylic acid, which reduces its hazardous features. Acrylamide processing occurs best at pH 7.5 and 40 degrees Celsius but retains stable for 13.37 hours when operating at 50 degrees Celsius [74].
- Acrylamide breakdown occurs through the enzyme in different foods such as bread alongside potato chips and cookies and meat products [74]. Through this method, the process creates safe food items, which preserve expected quality.

5.3 Combined processing strategies (HPP, blanching, coatings)

The food industry achieves effective acrylamide reduction in its products when different reduction methods are used in combination, ensuring both consumer safety and regulatory compliance. The fermentation process with certain probiotic strains, including *L. paracasei* and *B. breve*, results in substantial acrylamide reduction through their ability to break down acrylamide and its precursors [36, 75]. The optimization of fermentation parameters at their optimal levels requires adjustments of temperature, pH, and inoculum ratio. The optimization of cascara tea fermentation through Response Surface Methodology (RSM) resulted in enhanced antioxidant and microbial content according to previous studies [76].

The enzymatic approach, alongside asparaginase application in food production, provides an effective method to reduce acrylamide levels. Multiple enzymatic solutions used together could improve the effectiveness of acrylamide management systems. Research on acrylamide amidohydrolase demonstrates encouraging outcomes; however, scientists still need to verify how much acrylamide remains in food products and complete safety evaluations of enzymatic food processing methods.

Most producers obtain asparaginase from microbial fermentation processes. Various bacterial and fungal species such as *Bacillus sp.*, *Zymomonas mobilis*, and *Penicillium crustosum* have been optimized for large-scale enzyme production [77]. The development of enzyme engineering together with continuous fermentation techniques produced thermostable and pH-stable variants, which can be used for food processing applications [78, 79]. Acrylamide mitigation strategies become more effective when asparaginase works alongside additional food processing approaches.

- *Blanching*: Potato slices receive improved enzyme penetration after blanching, which results in acrylamide reductions reaching 90% [80].
- *High-Pressure Processing (HPP)*: The combination of HPP with enzymatic treatment enables better asparaginase penetration, thus resulting in acrylamide reduction levels between 26 and 47% [81].
- *Hydrocolloid coatings*: Zein-pectin coatings combined with asparaginase application result in more than 70% acrylamide reduction during the frying process of potato slices [82].

Enzymatic efficacy depends on multiple parameters that influence the outcome of asparaginase treatment for acrylamide mitigation.

- *Enzyme source and stability*: Variants of the enzyme that originate from *Mycobacterium gordonae* and *Pseudomonas sp.* demonstrate improved stability at extreme temperatures and pH values, leading to wider application opportunities across various food substrates [78, 79].
- *Enzyme dose and contact time*: Higher concentrations and longer exposure improve effectiveness. A treatment of potato slices with 84 U/mL purified asparaginase solution for 30 minutes eliminated detectable acrylamide levels [67].
- *Process conditions*: The maximum activity of the enzyme along with its ability to access the substrate requires specific conditions of temperature and pH and moisture content in the process.

Asparaginase presents a specific strategy for acrylamide minimization in plant food products. Implementing fermentation and processing workflows with pre-treatment and physical enhancement techniques creates opportunities for generating safer high-quality food products. Further industrial feasibility of the process is supported by ongoing research into enzyme stabilization and delivery systems despite present barriers including high costs and process changes.

6. Sensory, functional, and nutritional considerations

6.1 Volatile and non-volatile flavor compounds

Fermentation results in the creation of essential volatile compounds such as 1-hexanol and linalool that determine the aroma and flavor. Sensory evaluations demonstrate that different fermentation durations combined with LAB strains can produce unique taste and aroma profiles, which consumers find more appealing [25]. The research showed that 340 volatile compounds exist in fermented peppers, where specific compounds had substantial odor activity values, which improved sensory perception [39]. LAB fermentation of cereal products produces volatiles and non-volatiles, which affect sensory characteristics of fermented products. The compounds improve both nutritional value and safety, together with flavor and aroma properties, leading to better acceptance by consumers.

LAB fermentation results in the production of vital volatile compounds, which include acids along with alcohols and aldehydes that determine flavor characteristics. Acetic acid stands out for its sour taste, but other volatiles introduce sweet and malty flavors [83]. The research found 63 volatile compounds in fermented barley beverages that demonstrate fermentation's ability to generate diverse flavors. Non-volatile compounds, which include organic acids and amino acids, change during fermentation, leading to better nutritional value and improved product functions. Indole-3-lactic acid demonstrated significant growth in fermented barley beverages [84]. Non-volatile compounds such as Maillard-derived products determine the distinctive taste characteristics of sourdough bread, so they play an essential role in developing flavors [85]. The long-term health benefits and flavor stability of fermented cereal products depend heavily on non-volatile compounds, even though volatile compounds receive

more attention for their direct sensory effects, thus requiring a balanced approach for product development.

The combination of pH variations with volatile compound synthesis affects the flavor characteristics of fermented foods. The wild strain of *Lactobacillus plantarum* at 48 hours during Habanero pepper fermentation reached its highest levels of 3,3-dimethyl-1-hexanol and trans-2-hexene-1-al, which produced the most preferred odor. The final product's aroma and flavor receive their characteristics through pH changes during fermentation [86]. LAB fermentation of cereal products produces volatiles and non-volatiles, which affect the sensory characteristics of fermented products. The compounds improve both nutritional value and safety, together with flavor and aroma properties, leading to better acceptance by consumers. LAB-induced pH modifications of volatile compounds produce balanced flavor characteristics [87].

6.2 Managing trade-offs between taste and acrylamide reduction

The process of balancing acrylamide reduction with sensory qualities of plant-based foods during fermentation proves difficult to achieve. Plant-based products gain texture and flavor through fermentation, but this process could affect acrylamide concentrations and sensory quality. The fibrous structure of plant-based meat analogs becomes better during fermentation, yet prolonged fermentation causes unpleasant sliminess and decreased firmness. The enhancement of flavors through fermentation can sometimes produce unfavorable off-flavors that reduce sensory acceptance unless fermentation processes remain strictly controlled [88].

The combination of salt concentration optimization with pH value control during kohlrabi fermentation leads to enhanced sensory results. The combination of 3.5% NaCl solution at pH 4.2 provides optimal fermentation conditions, which produce acceptable quality along with superior sensory scores after 2 days of fermentation. The maintenance of sensory properties depends heavily on pH control because high temperatures tend to result in reduced product quality and decreased hardness [89]. The promising results of LAB and yeast combined for acrylamide reduction and bread quality improvement depend on the fermentation conditions and specific strains used. The inconsistent results between different bread batches might stem from the fermentation process.

The implementation of asparaginase as a method to decrease acrylamide content needs to be balanced with the preservation of product taste qualities [90]. The precise management of fermentation parameters enables the control of acrylamide formation alongside the preservation of sensory characteristics but demands exact monitoring to prevent product deterioration. Combining fermentation with proper management of processing parameters provides a solution to boost plant-based foods while reducing acrylamide risks. Recent studies also highlight the role of various compounds—such as cations, acids, and antioxidants—in further mitigating acrylamide formation in plant-based foods, especially cereals and potatoes [87, 91]. The complex relationships between sensory qualities and acrylamide formation require additional research to develop effective optimization methods.

6.3 LAB fermentation in plant-based alternatives

LAB fermentation of citrus vinegar by *Saccharomyces cerevisiae* together with *Lactobacillus plantarum* results in elevated levels of formic acid, lactic acid, and total

organic acids. The modification of pH along with organic acid composition generates improved antioxidant capacity and better sensory characteristics that include sweetness and umami taste along with flowery and fruity fragrance notes [92]. Dry-fermented sausages can maintain their probiotic *Lactobacillus plantarum* L125 viable cell counts throughout the storage duration. The fermentation process, which decreases pH levels, results in better sensory qualities of taste and texture without negatively affecting product quality [93].

Mixed fermentation of *Lactiplantibacillus plantarum* and *Levilactobacillus brevis* in traditional Chinese paocai leads to significant nitrite content reduction. The production of organic acids, especially lactic acid, functions as a key factor for nitrite destruction while simultaneously improving flavor characteristics. The fermentation process generates enhanced taste and aroma qualities through increased production of alcohols, esters, and acids [94].

Paocai fermentation through the application of homo-fermentative and hetero-fermentative LAB strains produces samples with different physicochemical features and flavor compositions. The fermentation process of LAB produces different amounts of lactic acid among homo-fermentative strains, but acetic acid production occurs in hetero-fermentative strains, which leads to divergent sensory outcomes. The metabolic processes between paocai samples prepared using homo-fermentative LAB and hetero-fermentative LAB show different patterns as demonstrated by Principal Component Analysis [95].

7. Conclusions

Plant-based foods benefit from lactic acid bacteria (LAB) fermentation as this technique effectively enhances food quality attributes as well as nutritional content. During LAB fermentation, multiple volatile and non-volatile substances are formed, such as alcohols acids, amino acids, and esters, which enhance sensorial properties and create appealing textures, thus gaining consumer popularity. Laboratory research found that fermentation time length together with selected specific strains and pH control determine the end sensory characteristics of fermented bread and cereal beverages as well as fermented vegetables.

LAB fermentation provides multiple effective methods to decrease acrylamide content in products using strategies that include modifying pH levels and removing asparagine and combining fermentation with yeast or using specific enzyme technologies. Strategies to optimize performance benefits alongside sensory quality maintenance or enhancement present themselves as complex optimization objectives. The production of unwanted textures alongside off-flavors occurs through fermentation processes that extend beyond normal ranges or when acids accumulate irregularly during the process.

Biotechnology experts leverage homo- and hetero-fermentative LAB strains as part of fermentation processes to manage safety aspects together with antioxidant potential and product taste in plant-based alternatives. The correct implementation of fermentation alongside strict parameter management enables balance between lowered acrylamide formation and sensory attributes that consumers appreciate.

Plant-based system production using LAB fermentation requires expertise in biological transformations together with instances of strain behavior and customer sensory demands. Additional interdisciplinary research needs to be conducted both to optimize processes and determine large-scale food industry solutions.

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Conflict of interest

The authors declare no conflict of interest.

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