

R E V I E W

L-Asparaginase potential in acrylamide mitigation from foodstuff: a mini-review

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Summary. L-Asparaginase (E.C. 3.5.1.1, LA) also known as L-asparagine amidohydrolase, specifically catalysis the breakdown of amino acid asparagine to aspartic acid and ammonia. It reduces the level of acrylamide that is produced during the baking of starchy foods. L-Asparaginase is widely distributed in animals, plants, and microorganisms. In food industries, acrylamide synthesized during the process of baking and frying at high temperatures under low moisture content during Maillard reaction, a non-enzymatic reaction. As acrylamide is a potential carcinogen, so strategies for its mitigation have significant importance on its level in the final product. Asparagine acts as a precursor in the process of acrylamide synthesis, so utilization of L-Asparaginase reduced its concentration in the final product. In this review, we have discussed the various pathways of acrylamide synthesis as well as different strategies for its elimination from final food products.

Keywords: L-Asparagine, carcinogen, Maillard reaction, elimination, Food

Introduction

L-Asparaginase (E.C. 3.5.1.1) has been proved to be a predominantly potential therapeutic agent for the treatment of different types of cancers especially acute lymphocytic leukemia (ALL) in children (1). In addition, L-asparaginase produces aspartate which acts as a precursor for the production of ornithine in urea cycle as well as acts as a precursor for the formation of oxaloacetate to generate glucose through gluconeogenic pathway within the human body (2). Moreover, L-asparaginase applications in food industries for the elimination of acrylamide from starchy food made it highly demanded agent in food processing. As enzymes are extensively distributed and could be isolated from indigenous sources including animals, plants, and

microorganisms. So, a large number of industrial processes in the area of environmental, industrial and food technology make use of enzymes at a range of stages (3). L-Asparaginase could be produced from a wide range of fungi, yeasts, bacteria, algae and actinomycetes in ample amount under different cultural conditions as well as could be isolated from various plants (4). L-Asparaginase from bacterial sources consists four identical subunits as tetrameric protein. Each subunit has 35.6 kDa mol. weight determined by x-ray crystallographically and each subunit have $C_{1377}H_{2208}N_{382}O_{442}S_{17}$ mol. formula (5).

Acrylamide ($CH_2=CH-CONH_2$; 2-propenamide), has 71.08 kDa molecular weight with non-volatile water-soluble characteristics of colorless solid crystals (6). Nitrile hydratase is used to hydrolyze

specifically acrylonitrile for the production of acrylamide at commercial level available both in monomeric as well as polymeric forms. Polymeric acrylamide as polyacrylamide have versatile application including the clarifying agent for drinking water; coagulant in wastewater treatment; applications in the construction of dam foundations and tunnels as well as used for the separation and purification of macromolecules in gel electrophoresis (7-9). The International Agency for Research on Cancer (IARC, 1994) has classified acrylamide as “probably carcinogenic to humans” on the basis of several studies. In 2002 Swedish National Food Administration (SNFA) was the first who recorded acrylamide contents present in food products. The European Commission Scientific Committee on Food (10) reported the intrinsic toxic effects of acrylamide like neurotoxic, genotoxic to somatic as well as to germ cells, carcinogenic effect and toxicity to reproductive organs. Recently an association between different types of cancers risk viz ovarian, breast, endometrial and renal, and intake of acrylamide has been evaluated repeatedly (11, 12).

L-Asparaginase has attracted the attention of food processing industries as a promising acrylamide mitigating agent (13) as treatment of starchy food with L-asparaginase decreases the synthesis of acrylamide before frying at high temperature (14). When asparagine-rich foods with starch contents are heated at high temperature these foods undergo a process called the Maillard reaction, this reaction is responsible for the induction of brown color, crust and toasted flavor in baked or fried foods. L-asparaginase transformed the asparagine into aspartic acid and ammonium before baking or frying the food and as a result, Maillard reaction takes place without the involvement of asparagine, and therefore production of acrylamide in a final food product is significantly reduced (15). Elimination of acrylamide completely from food products is impossible due to the presence of minor pathways which are independent on asparagine (16). L-asparaginase as a food processing aid of starchy foods can efficiently trim down the level of acrylamide upto 88% to 90% and advantage on other mitigation strategy is degradation of acrylamide would not affect the taste and appearance of the final product/products (17, 18). So in this review article, we have tried to describe L-

asparaginase, its sources as well as its role in reduction/mitigation of acrylamide, potential carcinogenic agent, from foods baked or fried at high temperature with low moisture content.

L-Asparaginase and its History

Normal body cells synthesized their own asparagine as a non-essential amino acid for cellular function with the help of asparagine synthetase and could also take this amino acid from blood circulation from extrinsic sources so normal body cells grow autonomous of its requirement. On the other hand, the tumor cells lack the ability to synthesize asparagine *in vivo* so they totally depend on the availability of high amount of this amino acid for their survival as well as for uncontrolled rapid proliferation (19). It was found first time in 1922 that guinea pig serum has higher activity of L-asparaginase and it was determined later on that it also possessed anti-neoplastic potential (20). Further, in 1953 Kidd (21) used mice and rats as an animal model to run the experimental trial for the evaluation of the anticancer potential of L-asparaginase isolated from guinea pig serum. After that in 1961 Broome (22) investigated L-asparaginase was responsible in the serum of guinea pig which significantly affects the proliferation of lymphoblastic cells due to its antineoplastic potential. Then in 1967 Oettgen and his colleague, first time determined the antineoplastic potential of this enzyme in humans with leukemia. After that application of L-asparaginase as an anti-acrylamide agent was reported in food industries as L-asparaginase degrades the asparagine present in foods especially in foods also having reducing sugars (23). So, during the baking procedure, L-asparaginase made asparagine unavailable in Maillard reaction mostly in potato products and French fries (24, 25).

Sources of L-Asparaginase

The requirements for the bulk amount of this enzyme for industrial applications both for pharmaceutical as well as for food industries have been motivated to find out new sources for L-asparaginase with high

Table 1. Different sources of L-Asparaginase production/isolation

Bacterial	Fungal	Plants
Pseudomonas aeruginosa (29)	<i>Aspergillus niger</i> (83)	<i>Pisum sativum</i> (85)
Bacillus subtilis (76)	<i>S. gulbargensis</i> (34)	<i>Soyabean leaves</i> (86)
Coliform bacteria (77)	<i>Penicillium spp</i> (32)	<i>Withania somnifera</i> (87)
E.coli (78)	<i>Aspergillus terreus</i> MTCC (84)	<i>Soyabean seeds</i> (88)
Bacillus sp (79)		<i>Amaranthus polygonoides</i> (89)
Halophilic bacteria (80)	Actinomycetes	Algae
<i>Pectobacterium caratovorum</i> (81)	<i>Actinomycete</i> strain RAF 10 (90)	<i>Chlamydomonas</i> (94)
<i>Proteus vulgaris</i> (82)	<i>Streptomyces albidoflavus</i> (91)	
<i>Serratia marcescens</i> (30)	<i>Streptomyces tendae</i> TKVL-333 (92)	Yeast
<i>Erwinia carotovora</i> (1)	<i>Streptomyces</i> ABR 2 (93)	<i>Pichia pastoris</i> (95)
Animals	<i>Streptomyces radiopugnans</i> MS1 (81)	<i>Rhodotorula</i> spp. (96)
Guinea pig serum (94)		<i>Candida utilis</i> (97)
		<i>Saccharomyces cerevisiae</i> (98)

specific activity and prolonged half-life. L-Asparaginases from various sources including plants (26, 27), bacteria (28-30), yeast and fungi (14, 31, 32) actinomycetes (33-35) algae and serum of some rodents (36, 37) have been isolated and characterized for therapeutic applications in the treatment of cancer as well as for food industries to reduce the production of acrylamide during baking/frying process (29), further Table.1 describes the sources of L-Asparaginase which could be used to reduce or mitigate the production of acrylamide in food industries.

Mechanism of acrylamide formation

Acrylamide is naturally absent from foods and is not added extrinsically but during the frying process, it synthesized in different ratios. Research studies indicated that foods frying, baking and roasting at high temperature above 120°C could be a vital source of acrylamide formation especially foods rich in carbohydrate (reducing sugars) particularly (38, 39).

In general, when foods are baked or processed at high temperature like potato products (24, 40) coffee (41), tea (42) bakery products (43) and others like roasted almonds, olives, dry fruits (44) contained the prominent concentration of acrylamide. Claus and his co-workers (2010) calculated that the average acrylamide intake in adults ranges from 0.3 to 0.6 mg/kg body weight per day while ingestion of acrylamide in children and adolescents is high per body weight ranges 0.4–0.6 mg/kg (43).

Acrylamide production follows various routes along the Maillard reaction system in final products of food and asparagine is the major one which produced highest quantity of acrylamide during this reaction in result of (6) asparagine reaction with reducing sugars primarily glucose and fructose (Figure 2) along some minor pathways (Figure 1) (45, 46). Maillard reaction is a reaction which is independent of any enzyme, the browning reaction occurred in foods in the result of aspecific combination of proteins, carbohydrates, and lipids on cooking or frying to inducing desirable color, aroma, and flavor (47). Asparagine, a non-essential amino acid found in potatoes in high concentration (93.6 mg/100 g) (46, 48), required carbohydrates for the production of acrylamide (49). Free asparagines level in rye varieties to be the main determinant of acrylamide production (50) and in baked flours and doughs (mainly rye and wheat) (51). The major limiting factors reported for acrylamide formation in potatoes are the reducing sugars (52) and asparagine in cereal products (53, 54). Molecular studies for tracking the construction of acrylamide structural constituents during frying revealed that three Carbon atoms and one Nitrogen atom are derived from asparagine molecule (45, 55).

In addition to asparagine and reducing sugars routes of acrylamide formation many minor acrylamide production pathways recommended via. acrolein and ammonia (Figure 1 & 2). Acrolein and ammonia have a very important role in the formation of acrylamide in lipid-rich foods. It was well reported that degradation of triglycerides (lipids) at high temperature

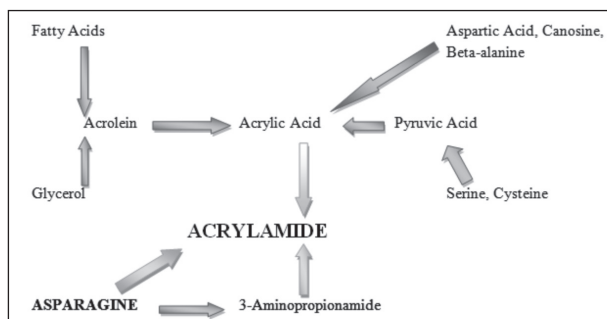


Figure 1. Basic pathways for the formation of acrylamide in foods designed and modified from(6).

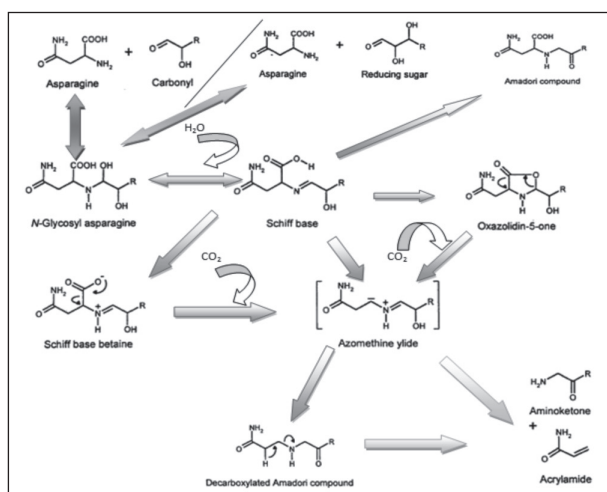


Figure 2. Formation of acrylamide from L-asparagine in the presence of α -hydroxycarbonyls/reducing sugars (Designed and modified from Stadler, Robert (15).

produced acrolein and acrylic acid as a precursor of acrylamide in food products (56). Further, the breakdown of amino acids with ammonia under low moisture content at high thermal conditions could produce acrylamide (57-59).

Amino acids including cysteine, aspartic acid, and glutamine have also been investigated to play a role in the formation of a small amount of acrylamide (Figure 1) (60, 61). However, it was reported that this route might be unrelated to the production of acrylamide in foods (62). Generally, the intensity of color directly related to the level of acrylamide in food products and production of acrylamide significantly increases towards the end of the frying process (63). The concentration of acrylamide produced in various foods and food products has also been further summarized below (Fig. 3).

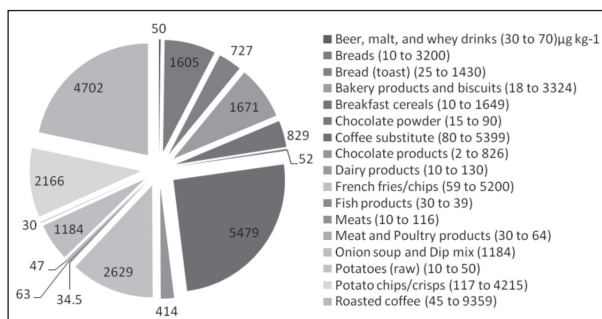


Figure 3. Concentration of acrylamide ($\mu\text{g kg}^{-1}$) in various foods/ food products produced on frying/baking (103, 106).

Mitigating agents of acrylamide

Considerable efforts for the elimination/reduction of acrylamide level in food products have been undertaken with the help of appropriate strategies. Reduction of acrylamide synthesis in food products could help to save the human being from food hazards as well as to generate awareness at household and industrial levels about the food safety. Numerous strategies for the mitigation of acrylamide so far regarding acrylamide formation at diverse stages of food production have been undertaken (Table 2) (64). Acrylamide mitigation strategies should involve elimination of asparagine alone, without affecting the other ingredients in the food; so that there would not be a significant difference in the sensorial properties of the food products (65). At large scale many approaches have been made to decrease and eradicate acrylamide levels by the addition of filamentous fungi like *A. oryzae* (66) antioxidants (67), compounds like allicin (68) and via applying different techniques based on genetic and agronomic manipulations for the reduction of the levels of the most important precursors of acrylamide like asparagine and sugars in produced plants (69, 70). The amalgamation of some additives though may help in the reduction of acrylamide, is to be expected to have a profound impact on the flavor and quality of the food products (71). Therefore, the use of L-asparaginase is relatively a simpler and effective solution, which makes it a potential contender for the reduction of acrylamide content in starchy fried food commodities in the food industry. L-Asparaginase pre-treatment of food materials before heat treatment is an appropriate solution suggested by several researchers (72, 73) as for the

Table 2. Effect of L-Asparaginase on acrylamide synthesis

Product	Reduction of Acrylamide conc. (%)	Preparation process/ Treatment technique	References
Potato product	>99	Frying	(45)
Cracker products	≈ 70	Dough Based	(99)
French fries	>90-99	Frying	(40)
Ginger biscuits, Semisweet biscuits, French fries, Sliced potato chips and Crispbread	34-92	Dough Based	(100)
Potato strips	≈ 80	frying/Immersion of Potato Strips	(101)
Potato strips	96	Frying	(102)
Sweet bread		Dough Based	(75)
Gingerbread	>97	Dough Based	(71)
Spanish rosquillas	Upto 90	Dough Based	(74)

reduction of free asparagine and thereby to minimize the imminent risk of synthesis of acrylamide as summarized in (Table 2). In addition, L-asparaginase pretreatment does not only leave the actual sensorial properties of the final food products unaffected but also enhances the flavor by increasing the glutamic acid content of the food. Further, L-asparaginase pretreatment reduced not only acrylamide but also the formation of hydroxymethylfurfural, a genotoxic intermediate of Millard reaction (74, 75). L-Asparaginase is marketed commercially as Acrylaway launched in 2007 by Novozymes, and as PreventASeL-asparaginase produced from *Aspergillus niger*, developed by DSM. Further research aimed at how to reduce the acrylamide contents in food products should proceed following the different strategies suggested (Table 3).

Conclusion

Acrylamide contents in foodstuff with well reported toxic effects including carcinogenicity, genotoxicity, reproductive & neurotoxicity are largely resulted from the reaction of R-amino group of the free amino acid asparagine with a carbonyl group(s) of reducing sugars such as glucose on applying heat as a major pathway along with some minor pathways. Strategies to reduce the concentration of L-asparagine and/or glucose content in unheated foods are expected to result in the reduction of acrylamide production in final food products. L-Asparaginase (E.C. 3.5.1.1, LA) as a potential

Table 3. Various strategies for Mitigation/Minimizing of Acrylamide content in processed food products

Strategies	References
1. Establish Databases of foods for	(103)
· Asparagine concentration	
· Glucose concentration	
2. Investigation of the relationship between	(103)
· Asparagine and acrylamide concentration in the processed and unprocessed foods	
3. Limiting the concentration of acrylamide precursors i.e Asparagine and glucose	(104)
a. Asparagine can be decreased by	
· Breeding for low asparagine biosynthesis	
· Suppressing genes that encode enzymes for asparagine synthesis	
· Selecting from available cultivars containing low conc. of asparagine	
b. Asparagine modification	
· Acetylation of Asparagine to N-acetyl-asparagine prevent acrylamide formation from N-glycoside intermediates	
4. Destruction and modification of acrylamide to make less toxic or harmless	(105)
a. Hydrolysis by	
· Acid and/ enzymes to degrade acrylamide formed in processed foods	
b. Polymerization of monomeric acrylamide by	
· Radiations, UV light	
· Free radical induced including one-electron oxidation intermediates of phenolic compounds and flavonoids (e.g., catechin, chlorogenic acid, tyrosine) Maillard browning products, tryptophan, and fatty acids	
c. Binding of acrylamide with SH-containing amino acids, esters, peptides, and proteins	
d. Decreasing synthesis by lowering pH using Citric acid in baking and frying formulations	

agent in food industries has promising applications with no well-reported side-effect for the elimination of acrylamide, generate during the baking of asparagine and starch-rich foods. Further, a number of strategies could be adopted to eliminate/reduce the acrylamide synthesis in food products but these required more research work.

Declaration of interest

The authors declare no competing interest regarding ownership and publication of this article.

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