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Review

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.

Key words: 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 aprecursor for the production of ornithine in urea cycle as well as acts as aprecursor 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 $C_{1377}H_{2208}N_{382}O_{442}S_{17}$ s mol. formula (5).

Acrylamide (CH_2=CH–$CONH_2$; 2-propenamide), has 71.08 kDa molecular weight with non-volatilewater-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 theconstruction 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 anassociation 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 afinal 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 described 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, thetumorcells 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 ananimal model to run the experimental trial for the evaluation of theanticancer potential of L-asparaginase isolated from guinea pig serum. After thatin 1961 Broome (22) investigated L-asparaginase was responsible in theserum 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 ananti-acrylamide agent was reported in food industries as L-asparaginase degrade 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 thebulk amount of this enzyme for industrial applications both for pharmaceutical as well as for foods industries have been motivated to find out new sources for L-asparaginase with high

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

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

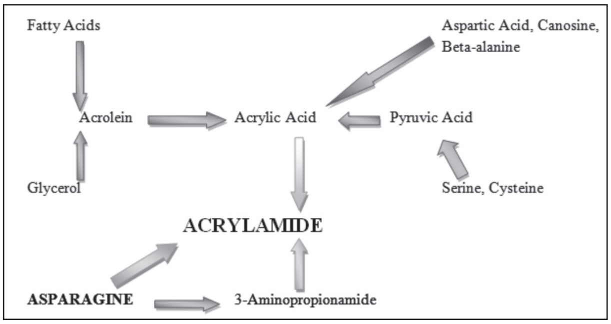

Figure 1. Basic pathways for the formation of acrylamide in foods designed and modified from(6).

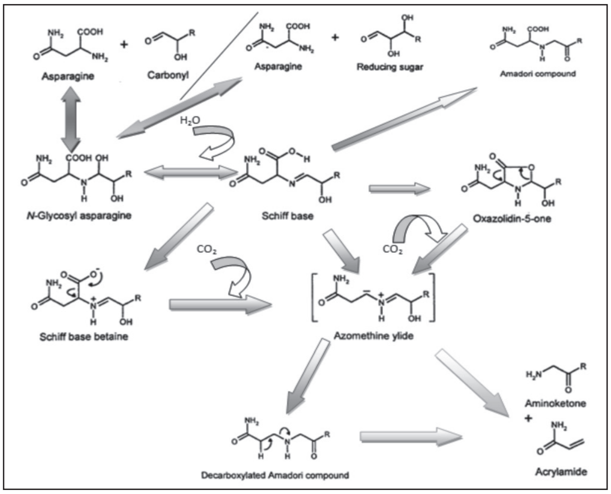

Figure 2. Formation of acrylamide from L-asparagine in the presence of α-hydroxycarbonyls/reducing sugars (Designed and modified from Stadler, Robert (15).

produced acrolein and acrylic acid as aprecursor of acrylamide in food products (56). Further, thebreakdown 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 role in the formation of asmall amount of acrylamide (Figure 1) (60, 61). However, it was reported that this route might be unrelated to theproduction of acrylamide in foods (62). Generally, theintensity 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).

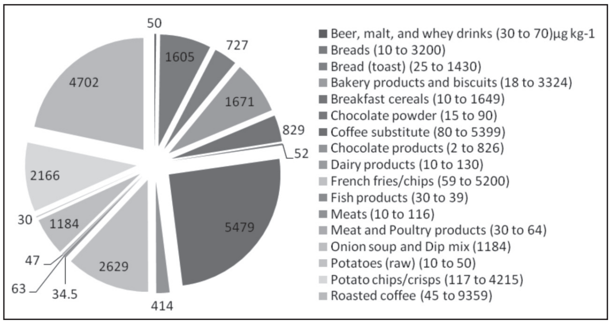

Figure 3. Concentration of acrylamide (μg kg-1) in various foods/ food products produced on frying/baking (103, 106).

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 asignificant 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 aprofound 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 thefood 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 *Aspergillusniger*, developed by DSM. Further research aimed at how to reducethe 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 arelargely 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 amajor 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 apotential

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

Strategies	References
1. Establish Databases of foods for · Asparagine concentration · Glucose concentration	(103)
2. Investigation of the relationship between · Asparagine and acrylamide concentration in the processed and unprocessed foods	(103)
3. Limiting the concentration of acrylamide precursors i.e Asparagine and glucose **a. Asparagine can be decreased by** · Breeding for low asparagines biosynthesis · Suppressing genes that encode enzymes for asparagines synthesis · Selecting from available cultivars containing low conc. of asparagine **b. Asparagine modification** · Acetylation of Asparagine to *N*-acetylasparagine prevent acrylamide formation from*N*-glycoside intermediates	(104)
4. Destruction and modification of acrylamide to make less toxic or harmless **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**	(105)

agent in food industries has promising applications with no well-reportedside-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.

References

1. Abakumova OY, Podobed OV, Karalkin PA, Kondakova LI, Sokolov NN. Antitumor activity of L-asparaginase from Erwinia carotovora against different human and animal leukemic and solid tumor cell lines. Biochemistry (Moscow) Supplement Series B: Biomedical Chemistry 2012; 6(4): 307-16.
2. Hosamani R, Kaliwal B. L-asparaginase an anti-tumor agent production by Fusarium equiseti using solid state fermentation. International Journal of Drug Discovery 2011; 3(2): 88-99.
3. Munir N, Asgher M, Tahir IM, Riaz M, Bilal M, Shah SA. Utilization of agro-wastes for production of ligninolytic enzymes in liquid state fermentation by Phanerochaete chrysosporium-IBL-03. IJCBS 2015; 7: 9-14.
4. Yadav S, Verma SK, Singh J, Kumar A. Industrial production and clinical application of L-asparaginase: A chemotherapeutic agent. Stroke 2014; 76: 41.
5. Fullmer A, O'Brien S, Kantarjian H, Jabbour E. Emerging therapy for the treatment of acute lymphoblastic leukemia. Expert Opinion on Emerging Drugs 2010; 15(1): 1-11.
6. Eriksson S. Acrylamide in food products: Identification, formation and analytical methodology. 2005.
7. Manson J, Brabec MJ, Buelke-Sam J, Carlson GP, Chapin RE, Favor JB, et al. NTP-CERHR Expert Panel report on the reproductive and developmental toxicity of acrylamide. Birth Defects Res B: Dev Reprod Toxicol 2005; 74(1): 17-113.
8. Shipp A, Lawrence G, Gentry R, McDonald T, Bartow H, Bounds J, et al. Acrylamide: Review of Toxicity Data and Dose-Response Analyses for Cancer and Noncancer Effects. Crit Rev Toxicol 2006; 36(6-7): 481-608.
9. Mojska H, Gielecińska I, Szponar L, Ołtarzewski M. Estimation of the dietary acrylamide exposure of the Polish population. Food Chem Toxicol 2010; 48(8-9): 2090-6.
10. Lindsay SJ, inventor; Lindsay, S J (LIND-Individual), assignee. Hand-held pneumatic impact handpiece for delicate, e.g. hand engraving operation, of jewelry, has variable fuel meter and work energy supply which regulate supply of pressurized fluid and of work energy based on pressure sensor output. US patent US6691798-B1. 2004 February 17.
11. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA. A Prospective Study of Dietary Acrylamide Intake and the Risk of Endometrial, Ovarian, and Breast Cancer. Cancer Epidemiology Biomarkers & Prevention 2007; 16(11): 2304-13.
12. Thonning Olesen P, Olsen A, Frandsen H, Frederiksen K, Overvad K, Tjønneland A. Acrylamide exposure and incidence of breast cancer among postmenopausal women in the Danish Diet, Cancer and Health Study. Int J Cancer 2008; 122(9): 2094-100.
13. Gökmen V, Palazoğlu TK, enyuva HZ. Relation between the acrylamide formation and time–temperature history of surface and core regions of French fries. J Food Eng 2006; 77(4): 972-6.
14. Baskar G, Renganathan S. Production of L-Asparaginase from Natural Substrates by Aspergillus terreus MTCC 1782: Effect of Substrate, Supplementary Nitrogen Source and L-Asparagine. International Journal of Chemical Reactor Engineering 2009; 7(1).
15. Stadler RH, Robert F, Riediker S, Varga N, Davidek T, Devaud S, et al. In-Depth Mechanistic Study on the Formation of Acrylamide and Other Vinylogous Compounds by the Maillard Reaction. J Agric Food Chem 2004; 52(17): 5550-8.
16. Kornbrust BA, Stringer MA, Lange NEK, Hendriksen HV, Whitehurst R, Oort M. Asparaginase-an enzyme for acrylamide reduction in food products. Enzymes in food technology 2010; 2: 59-87.
17. Lineback DR, Coughlin JR, Stadler RH. Acrylamide in Foods: A Review of the Science and Future Considerations. Annual Review of Food Science and Technology 2012; 3(1): 15-35.
18. Medeiros Vinci R, Mestdagh F, De Meulenaer B. Acrylamide formation in fried potato products – Present and future, a critical review on mitigation strategies. Food Chem 2012; 133(4): 1138-54.
19. Pieters R, Hunger SP, Boos J, Rizzari C, Silverman L, Baruchel A, et al. L'asparaginase treatment in acute lymphoblastic leukemia. Cancer 2011; 117(2): 238-49.
20. Clementi A. La Désamidation Enzymatique De L'asparagine Chez Les Différentes Espéces Animales Et La Signification Physio Logique De Sa Presence Dans L'organisme. Archives Internationales de Physiologie 1922; 19(4): 369-98.
21. Kidd JG. Regression of transplanted lymphomas induced in vivo by means of normal guinea pig serum: i. Course of transplanted cancers of various kinds in mice and rats given guinea pig serum, horse serum, or rabbit serum. J Exp Med 1953; 98(6): 565-82.
22. Broome JD. Evidence that the L-Asparaginase Activity of Guinea Pig Serum is responsible for its Antilymphoma Effects. Nature 1961; 191(4793): 1114-5.
23. Oettgen HF, Old L, Boyse E, Campbell H, Philips F, Clarkson B, et al. Inhibition of leukemias in man by L-asparaginase. Cancer Res 1967; 27(12): 2619.
24. Tareke E, Rydberg P, Karlsson P, Eriksson S, Törnqvist M. Analysis of Acrylamide, a Carcinogen Formed in Heated Foodstuffs. J Agric Food Chem 2002; 50(17): 4998-5006.
25. El-Ghonemy DH. Microbial amidases and their industrial

applications: a review. Journal of Medical Microbiology & Diagnosis 2015; 4(1): 1.

26. Bell TL, Adams MA. Ecophysiology of ectomycorrhizal fungi associated with Pinus spp. in low rainfall areas of Western Australia. Plant Ecology (formerly Vegetatio) 2004; 171(1/2): 35-52.
27. Oza VP, Parmar PP, Patel DH, Subramanian RB. Cloning, expression and characterization of l-asparaginase from Withania somnifera L. for large scale production. 3 Biotech 2011; 1(1): 21-6.
28. Borkotaky B, Bezbaruah R. Production and properties of asparaginase from a newErwinia sp. Folia Microbiol (Praha) 2002; 47(5): 473-6.
29. El-Bessoumy AA, Sarhan M, Mansour J. Production, Isolation, and Purification of L-Asparaginase from Pseudomonas Aeruginosa 50071 Using Solid-state Fermentation. BMB Reports 2004; 37(4): 387-93.
30. Agarwal A, Kumar S, Veeranki VD. Effect of chemical and physical parameters on the production of l-asparaginase from a newly isolated Serratia marcescens SK-07. Lett Appl Microbiol 2011; 52(4): 307-13.
31. Sarquis MIdM, Oliveira EMM, Santos AS, Costa GLd. Production of L-asparaginase by filamentous fungi. Mem Inst Oswaldo Cruz 2004; 99(5): 489-92.
32. Soniyamby A, Lalitha S, Praveesh B, Priyadarshini V. Isolation, production and anti-tumor activity of L-asparaginase of Penicillium sp. International Journal of Microbiological Research 2011; 2(1): 38-42.
33. Dhevagi P, Poorani E. L-Asparaginase from Marine Actinomycetes of Thoothukudi Coastal Ecosystem. International Journal of Current Microbiology and Applied Sciences 2016; 5(7): 295-306.
34. Amena S, Vishalakshi N, Prabhakar M, Dayanand A, Lingappa K. Production, purification and characterization of L-asparaginase from Streptomyces gulbargensis. Braz J Microbiol 2010; 41(1): 173-8.
35. Hu H, Lin H-P, Xie Q, Li L, Xie X-Q, Sun M, et al. Streptomyces shenzhenensis sp. nov., a novel actinomycete isolated from mangrove sediment. Antonie Van Leeuwenhoek 2011; 100(4): 631-7.
36. Iyer P, Singhal RS. Production of glutaminase (E.C.3.2.1.5) from Zygosaccharomyces rouxii: Statistical optimization using response surface methodology. Bioresour Technol 2008; 99(10): 4300-7.
37. Sathish T, Prakasham RS. Enrichment of glutaminase production by Bacillus subtilis RSPŕGLU in submerged cultivation based on neural network—genetic algorithm approach. J Chem Technol Biotechnol 2010; 85(1): 50-8.
38. Becalski A, Lau BPY, Lewis D, Seaman SW. Acrylamide in Foods: Occurrence, Sources, and Modeling. J Agric Food Chem 2003; 51(3): 802-8.
39. Lineback DR, Coughlin JR, Stadler RH. Acrylamide in foods: a review of the science and future considerations. Annual review of food science and technology 2012; 3: 15-35.
40. Pedreschi F, Kaack K, Granby K. Reduction of acrylamide formation in potato slices during frying. LWT - Food Science and Technology 2004; 37(6): 679-85.
41. Granby K, Fagt S. Analysis of acrylamide in coffee and dietary exposure to acrylamide from coffee. Anal Chim Acta 2004; 520(1-2): 177-82.
42. Mizukami Y, Kohata K, Yamaguchi Y, Hayashi N, Sawai Y, Chuda Y, et al. Analysis of Acrylamide in Green Tea by Gas Chromatography–Mass Spectrometry. J Agric Food Chem 2006; 54(19): 7370-7.
43. Claus A, Schieber A, Spitzner D, Carle R, Halford N, Mottram D. Thermal formation of acrylamide and impact of agronomic and technological factors on its minimization in bakery products. Acrylamide: Influence of plant genetics, agronomy and food processing 2009: 3.
44. Amrein TM, Andres L, Escher F, Amadò R. Occurrence of acrylamide in selected foods and mitigation options. Food Addit Contam 2007; 24(sup1): 13-25.
45. Zyzak DV, Sanders RA, Stojanovic M, Tallmadge DH, Eberhart BL, Ewald DK, et al. Acrylamide Formation Mechanism in Heated Foods. J Agric Food Chem 2003; 51(16): 4782-7.
46. Yaylayan VA, Stadler RH. Acrylamide formation in food: A mechanistic perspective. J AOAC Int 2005; 88(1): 262-7.
47. Tareke E, Rydberg P, Karlsson P, Eriksson S, Törnqvist M. Acrylamide: A Cooking Carcinogen? Chem Res Toxicol 2000; 13(6): 517-22.
48. Martin FL, Ames JM. Formation of Strecker Aldehydes and Pyrazines in a Fried Potato Model System. J Agric Food Chem 2001; 49(8): 3885-92.
49. Pedreschi F, León J, Mery D, Moyano P, Pedreschi R, Kaack K, et al. Color development and acrylamide content of predried potato chips. J Food Eng 2007; 79(3): 786-93.
50. Curtis TY, Powers SJ, Balagiannis D, Elmore JS, Mottram DS, Parry MAJ, et al. Free Amino Acids and Sugars in Rye Grain: Implications for Acrylamide Formation. J Agric Food Chem 2010; 58(3): 1959-69.
51. Hamlet CG, Sadd PA, Liang L. Correlations between the Amounts of Free Asparagine and Saccharides Present in Commercial Cereal Flours in the United Kingdom and the Generation of Acrylamide during Cooking. J Agric Food Chem 2008; 56(15): 6145-53.
52. Sanny M, Jinap S, Bakker EJ, van Boekel MAJS, Luning PA. Is lowering reducing sugars concentration in French fries an effective measure to reduce acrylamide concentration in food service establishments? Food Chem 2012; 135(3): 2012-20.
53. Amrein TM, Schönbächler B, Escher F, Amadò R. Acrylamide in Gingerbread: Critical Factors for Formation and Possible Ways for Reduction. J Agric Food Chem 2004; 52(13): 4282-8.
54. Claus A, Carle R, Schieber A. Acrylamide in cereal products: A review. Journal of Cereal Science 2008; 47(2): 118-33.
55. Stadler RH, Blank I, Varga N, Robert F, Hau J, Guy PA, et al. Food chemistry: Acrylamide from Maillard reaction products. Nature 2002; 419(6906): 449-50.
56. Gertz C, Klostermann S. Analysis of acrylamide and mech-

anisms of its formation in deep-fried products. Eur J Lipid Sci Technol 2002; 104(11): 762-71.

57. Becalski A, Lau BPY, Lewis D, Seaman SW, Hayward S, Sahagian M, et al. Acrylamide in French Fries: Influence of Free Amino Acids and Sugars. J Agric Food Chem 2004; 52(12): 3801-6.
58. Hidalgo FJ, Zamora R. Conversion of Phenylalanine into Styrene by 2,4-Decadienal in Model Systems. J Agric Food Chem 2007; 55(12): 4902-6.
59. Zamora R, Gallardo E, Hidalgo FJ. Strecker Degradation of Phenylalanine Initiated by 2,4-Decadienal or Methyl 13-Oxooctadeca-9,11-dienoate in Model Systems. J Agric Food Chem 2007; 55(4): 1308-14.
60. Sohn M, Ho C-T. Ammonia Generation during Thermal Degradation of Amino Acids. J Agric Food Chem 1995; 43(12): 3001-3.
61. Ezeji TC, Groberg M, Qureshi N, Blaschek HP. Continuous Production of Butanol from Starch-Based Packing Peanuts. Biotechnology for Fuels and Chemicals: Springer Nature; 2003. p. 375-82.
62. Weisshaar R. Acrylamide in bakery products-Results from model experiments. Deutsche Lebensmittel-Rundschau 2004; 100(3): 92-+.
63. Xu Y, Cui B, Ran R, Liu Y, Chen H, Kai G, et al. Risk assessment, formation, and mitigation of dietary acrylamide: Current status and future prospects. Food Chem Toxicol 2014; 69: 1-12.
64. Visvanathan R KT. Acrylamide in Food Products: A Review. Journal of Food Processing & Technology 2014; 05(07).
65. Elmore JS, Parker JK, Halford NG, Muttucumaru N, Mottram DS. Effects of Plant Sulfur Nutrition on Acrylamide and Aroma Compounds in Cooked Wheat. J Agric Food Chem 2008; 56(15): 6173-9.
66. Wakaizumi M, Yamamoto H, Fujimoto N, Ozeki K. Acrylamide degradation by filamentous fungi used in food and beverage industries. J Biosci Bioeng 2009; 108(5): 391-3.
67. Ou S, Shi J, Huang C, Zhang G, Teng J, Jiang Y, et al. Effect of antioxidants on elimination and formation of acrylamide in model reaction systems. J Hazard Mater 2010; 182(1-3): 863-8.
68. Yuan Y, Shu C, Zhou B, Qi X, Xiang J. Impact of selected additives on acrylamide formation in asparagine/sugar Maillard model systems. Food Res Int 2011; 44(1): 449-55.
69. Curtis TY, Muttucumaru N, Shewry PR, Parry MAJ, Powers SJ, Elmore JS, et al. Effects of Genotype and Environment on Free Amino Acid Levels in Wheat Grain: Implications for Acrylamide Formation during Processing. J Agric Food Chem 2009; 57(3): 1013-21.
70. Halford NG, Muttucumaru N, Curtis TY, Parry MAJ. Genetic and agronomic approaches to decreasing acrylamide precursors in crop plants. Food Addit Contam 2007; 24(sup1): 26-36.
71. Ciesarova Z, Kukurova K, Bednáriková A, Marková L, Baxa S. Improvement of cereal product safety by enzymatic way of acrylamide mitigation. Czech J Food Sci 2009; 27: S96-S8.
72. Pedreschi F, Kaack K, Granby K. The effect of asparaginase on acrylamide formation in French fries. Food Chem 2008; 109(2): 386-92.
73. Xu F, Oruna-Concha M-J, Elmore JS. The use of asparaginase to reduce acrylamide levels in cooked food. Food Chem 2016; 210: 163-71.
74. KukurovÃ¡ Kn, Morales FJ, BednÃ¡rikovÃ¡ A, CiesarovÃ¡ Z. Effect of L-asparaginase on acrylamide mitigation in a fried-dough pastry model. Mol Nutr Food Res 2009; 53(12): 1532-9.
75. Kumar NM, Shimray CA, Indrani D, Manonmani H. Reduction of acrylamide formation in sweet bread with l-asparaginase treatment. Food and bioprocess technology 2014; 7(3): 741-8.
76. Mazini M. L-Asparaginase activity by various bacteria. Journal of Missan Researches 2007; 4(7): 1-9.
77. Shah AJ, Karadi RV, Parekh PP. Isolation, Optimization and Production of L-asparaginase from Coliform Bacteria. Asian Journal of Biotechnology 2010; 2(3): 169-77.
78. Abbas AA, Sabbah MA, Kathum OA. Partial purification and cytotoxic activity of L-asparaginase isolated from Escherichia coli. Iraq J Sci 2010; 51(2): 290-4.
79. Maysa E, Amira M, Gamal E, Sanaa T, Sayed E. Production, immobilization and anti-tumor activity of L-asparaginase of Bacillus sp R36. Journal of American Science 2010; 6(8): 157-65.
80. Ebrahiminezhad A, Rasoul-Amini S, Ghasemi Y. L-asparaginase production by moderate halophilic bacteria isolated from Maharloo salt lake. Indian J Microbiol 2011; 51(3): 307-11.
81. Kumar S, Dasu VV, Pakshirajan K. Studies on pH and thermal stability of novel purified L-asparaginase from Pectobacterium carotovorum MTCC 1428. Microbiology 2011; 80(3): 355-62.
82. Toma RJ, Suo'd AM, Hassan SA, Aon MAA, Salman SK. Extraction and purification of L-Asparaginase II from local isolate of Proteus vulgaris. 2011.
83. Mishra A. Production of L-Asparaginase, an Anticancer Agent, From Aspergillus niger Using Agricultural Waste in Solid State Fermentation. Appl Biochem Biotechnol 2006; 135(1): 33-42.
84. Baskar G, Renganathan S. Statistical and evolutionary optimisation of operating conditions for enhanced production of fungal l-asparaginase. Chemical Papers 2011; 65(6).
85. Chagas EP, Sodek L. Purification and properties of asparaginase from the testa of immature seeds of pea (Pisum sativum L.). Brazilian Archives of Biology and Technology 2001; 44(3): 239-45.
86. Cho C, Lee H, Chung E, Kim KM, Heo JE, Kim J, et al. Molecular characterization of the soybean L-asparaginase gene induced by low temperature stress. Mol Cells 2007; 23(3): 280.
87. Verma N, Kataria M, Kumar K, Saini J. Comparative study of L-asparaginase from different cytotypes of Withania somnifera (L.) Dunal and its purification. J Nat Prod Plant Resour 2012; 2(4): 475-81.

88. Pandurangan S, Pajak A, Molnar SJ, Cober ER, Dhaubhadel S, Hernandez-Sebastia C, et al. Relationship between asparagine metabolism and protein concentration in soybean seed. J Exp Bot 2012; 63(8): 3173-84.
89. Naveena B, Narayani T, Sakthiselvan P, Partha N. Antioxidant and antimicrobial efficacies of Amaranthus polygonoides and its impact on L-asparaginase production. African Journal of Biotechnology 2012; 11(61): 12483-90.
90. Rabah FL, Elshafei A, Saker M, Cheikh B, Hocine H. Screening, Isolation and Characterization of a Novel Antimicrobial Producing Actinomycete, Strain RAF10. Biotechnology(Faisalabad) 2007; 6(4): 489-96.
91. Narayana KJP, Kumar KG, Vijayalakshmi M. L-asparaginase production by Streptomyces albidoflavus. Indian J Microbiol 2008; 48(3): 331-6.
92. Kavitha A, Prabhakar P, Narasimhulu M, Vijayalakshmi M, Venkateswarlu Y, Venkateswara Rao K, et al. Isolation, characterization and biological evaluation of bioactive metabolites from Nocardia levis MK-VL_113. Microbiol Res 2010; 165(3): 199-210.
93. Sudhir AP, Dave BR, Trivedi KA, Subramanian RB. Production and amplification of an l-asparaginase gene from actinomycete isolate Streptomyces ABR2. Annals of Microbiology 2012; 62(4): 1609-14.
94. Bano M, Sivaramakrishnan VM. Preparation and properties of L-asparaginase from green chillies (Capsicum annum L.). J Biosci 1980; 2(4): 291-7.
95. Ferrara MA, Severino NMB, Mansure JJ, Martins AS, Oliveira EMM, Siani AC, et al. Asparaginase production by a recombinant Pichia pastoris strain harbouring Saccharomyces cerevisiae ASP3 gene. Enzyme Microb Technol 2006; 39(7): 1457-63.
96. Lapmak K, Lumyong S, Thongkuntha S, Wongputtisin P, Sardsud U. L-asparaginase production by Bipolaris sp. BR438 isolated from brown rice in Thailand. 2010.
97. Momeni V, Alemzadeh I, Vosoughi M. Enhancement of L-asparaginase Production by Candida utilis in a 13 L Fermenter and its Purification. International Journal of Engineering-Transactions B: Applications 2015; 28(8): 1134.
98. Facchinetti de Castro Girão L, Gonçalves da Rocha SL, Sobral RS, Dinis Ano Bom AP, Franco Sampaio AL, Godinho da Silva J, et al. Saccharomyces cerevisiae asparaginase II, a potential antileukemic drug: Purification and characterization of the enzyme expressed in Pichia pastoris. Protein Expr Purif 2016; 120: 118-25.
99. Vass M, Amrein T, Schönbächler B, Escher F, Amado R. Ways to reduce the acrylamide formation in cracker products. Czech Journal of food sciences 2004; 22: 19-21.
100. Hendriksen HV, Kornbrust BA, Østergaard PR, Stringer MA. Evaluating the Potential for Enzymatic Acrylamide Mitigation in a Range of Food Products Using an Asparaginase from Aspergillus oryzae. J Agric Food Chem 2009; 57(10): 4168-76.
101. Mahajan RV, Saran S, Kameswaran K, Kumar V, Saxena RK. Efficient production of l-asparaginase from Bacillus licheniformis with low-glutaminase activity: Optimization, scale up and acrylamide degradation studies. Bioresour Technol 2012; 125: 11-6.
102. Kumar NM, Manonmani H. Purification, characterization and kinetic properties of extracellular l-asparaginase produced by Cladosporium sp. World J Microbiol Biotechnol 2013; 29(4): 577-87.
103. Friedman M. Chemistry, biochemistry, and safety of acrylamide. A review. J Agric Food Chem 2003; 51(16): 4504-26.
104. Friedman M, Brandon DL. Nutritional and Health Benefits of Soy Proteins†. J Agric Food Chem 2001; 49(3): 1069-86.
105. Masi C, Dinnella C, Barnabà M, Navarini L, Monteleone E. Sensory Properties of Under-Roasted Coffee Beverages. J Food Sci 2013; 78(8): S1290-S300.
106. Elder VA, Fulcher JG, Leung HK-H, Topor MG. Method for reducing acrylamide formation in thermally processed foods. Google Patents; 2007.

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