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Short Review on Microbial L- methioninase and its Applications

Marwa A. Yassin[1*], Ahmed Shindia[1], , Mohamed Soud[1], Ashraf S.A. El-Sayed[1*] ,Esraa azab[2*]

[1]Enzymology and Fungal Biotechnology Lab (EFBL), Botany and Microbiology Department, Faculty of Science, Zagazig University, Zagazig 44519, Egypt
[2]Zoonotic Diseases Department, Faculty of Veterinary Medicine, Zagazig University

ABSTRACT **: L-Asparaginase is an effective chemotherapeutic enzyme catalyzes hydrolysis of the amino acid asparagine into aspartate and ammonia which is essential for tumour cells. It has been effectively used in the treatment of acute lymphoblastic leukemia and lymphosarcoma. Acrylamide, the low molecular weight human carcinogen, has neurotoxic and genotoxic effects. The mechanism behind the inhibition of acrylamide generation in food systems are the hydrolysis of carboxamide group of amine in asparagine into asparatic acid and this lead to either elimination or reduction in acrylamide content in food. Utilizing of L-asparaginase as a mitigation technique for reduced acrylamide concentration is the main promising application in food industry. The present review focuses on enzyme sources, mechanism of action as well as a detailed information about its production and purification. It also provides some recent updated information about its chemical structure, physiochemical, kinetic properties of the enzyme and its applications in food industry.**

KEYWORDS: Asparaginase; chemotherapeutic; physiochemical; kinetic; Acrylamide.

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I. INTRODUCTION

Enzyme technology has allowed scientists to utilize, modify, and improve the efficiency of enzymes to improve their maximum function and efficacy (Offman et al., 2010; Savitri et al., 2003). The microbial lasparaginase (lasparaginamide hydrolase, E.C. 3.5.1.1) is widely used and has been studied for its potential as a carcinogen (Benchamin et al., 2019). Lasparaginase has many uses in the pharmaceutical, healthcare and food industries (Cachumba et al., 2016). Many studies have shown that rasparagine acts as an anticarcinogen because of the potential and chemical efficiency of converting asparagine (present in cancer cells above normal levels) to ammonia and aspartic acid. (Ray et al., 2019). Therefore, rasparaginase is an unavoidable tool for the treatment of acute malignancies of the lymphatic system and melanoma (Dias et al., 2016). Lasparaginase suppresses the growth of cancer cells by reducing lasparagin (a non-essential amino acid), which is an essential nutrient for malignant cells and cancer cells. Lasparagnase is mainly produced by different microorganisms that inhabit different ecosystems & # 40; Shukla et al. , 2014 & # 41 ;. Depending on the localization of rasparaginase in the cell, it can be divided into two isoforms, rasparaginase I and II. This allows these two forms to be genetically distinguished from each other, and rasparaginases from different microorganisms have different properties. In nature, rasparaginase occurs in a variety of structural forms, including dimers, tetramers, and hexamers. Asparaginase can actively degrade acrylamide (C3H5NO), a small hydrophilic molecule that is a potential human carcinogen. Acrylamide was classified as a carcinogen by the International Agency for Research on Cancer (IARC) in 1994. In 2000, the WHO (World Health Organization) upheld the IARC's decision to declare

acrylamide as a carcinogenic molecule (Krishnakumar and Visvanathan 2014). Acrylamide is found in high-carbohydrate foods that are cooked at high temperatures (9). Acrylamide is formed by a mirrored reaction that occurs at extreme temperatures between asparagine and carbonyl compounds. The temperature for acrylamide production usually exceeds 100 ° C (Alam et al., 2019). Acrylamide is found in baked goods, fried foods and starchy foods because asparagine is the main cause of acrylamide formation. Lasparagnase neutralizes the production of acrylamide (Xu et al., 2016)

There are several sources for the isolation and purification of lasparaginase, but for commercial purposes it is currently isolated from two major bacterial sources. Lasparagnase is present in most bacteria, fungi, plant tissues, algae, and some animals. Rodents have rasparaginase in their serum, and humans lack this important enzyme (12). The rasparaginase enzyme is considered to be the most important enzyme for its role in lymphoblast therapy and its inhibition of acrylamide production as a side reaction during industrial food processing (Narta et al., 2007). The importance of this enzyme is also demonstrated by its high global demand, which reached US $ 380 million in 2017 and is projected to increase to US $ 420 million by 2025 (10). Due to the important health and industrial importance of this enzyme, rasparaginase is currently being investigated for its powerful role in various types of cancer, including breast cancer (14). This review focused on the use of rasparaginase enzyme technology in the healthcare and food industry and expanded shortly thereafter. We have summarized the various enzyme sources, their physicochemical and kinetic properties, and their wide range of uses.

1.1. Source of LAsparaginase:

The presence of rasparaginase has been studied in a variety of organisms, including non-human animals, plants and microorganisms (bacteria, fungi, algae, yeasts, actinomycetes). Lasparaginase is found in various groups of animals and plants, but other possible sources (bacteria, fungi, algae, yeasts, actinomycetes) are being investigated by researchers for painstaking extraction strategies (" table 1). Obtaining enzymes from living organisms on a large scale is much easier due to their simple manufacturing process. Here, we have described various microbial sources of lasparaginase.

Table 1: Microbial species producing L-asparaginase

Sources	Organisms	Reference
Bacteria	Acinetobacter	(15)
	Calcoaceticus	(Tiwari and Dua, 1996)
	Azotobacter agilis	(16)
	Brevibacillus brevis	(17)
	Citrobacter sp.	(18)
	Escherichia coli	(Microbiologica 1977,)
	Enterobacter aerogenes	(20)
	E. cloacae	(21)
	Erwinia aroideae	(Tiwari and Dua, 1996)
	E. cartovora	(23)
	E. chrysanthemi	(24)
	Helicobacter pylori	(25)
	Klebsiella pneumonia	(17)
	Pectobacterium Carotovorum	(Kotzia and Labrou, 2009)
	Pseudomonas sp	(27)
	P. fluorescens AG	(28)
	P. geniculate	(15)
	P. ovalis	(15)
	P. stutzeri	(29)
	Pyrococcus horikoshii	(30)
	Serratia marcescens	(27)
	Thermus thermophiles	(Pritsa and Kyriakidis, 2001)
	T. aquaticus	(32)
	Vibrio succinogenes	(33)
	Citrobacter freundi	(34)
	Bacillus circulans	(35)

Continued Table. 1	B. coagulans	(36)
	Bacillus sp.	(37)
	B. mesentericus	(Kotzia and Labrou, 2009)
	B. polymyxa	(38)
	B. subtilus	(38)
	B. licheniformis	(Fisher and Wray, 2002)
	B. circulans MTCC 8574	(40)
	Corynebacterium glutamicum	(41)
	Mycobacterium bovis	(15)
	M. phlei	(34)
	Staphylococcus sp.	(17)
	S. aureus	(42)
	Streptococcus albus	(33)
	Candida utilis	(43)
Yeast	C. guilliermondii	(44)
	C. bombicola	(Daverey and Pakshirajan, 2010)
	Rhodotorula sp.	(Daverey and Pakshirajan, 2010)
	Alternaria sp	(46)
Fungi	Aspergillus nidulans	(47)
	A. niger	(44)
	A. tamari	(48)
	A. oryzae	(49)
	A. terreus	(50)
	Cylidrocapron obtusisporum	(50)
	Mucor sp.	(Mohapatra and Bapuji, 1997)
	Fusarium roseum	(52)
	A.fumigatus	(Mishra, 2006)
	Actinomyces sp	(53)
Actinomycetes	Streptomyces albidoflavus	(54)
	S. aurantiacus	(54)
	S. collinus	(55)
	S. griseus	(56)
	S. gulbargensis	(Amena et al., 2010)
	S. karnatakensis	(58)
	S. longsporusflavus	(59)

1. Physiochemical properties of l-asparaginases:

Physiochemical properties of few microbial l-asparaginases are summarized in Table 2. It can be mentioned that most of the microbial strains have optimum temperature variety of 37–40 °C however the strains which includes Thermococcus kodaka TK1656 and Thermococcus gammatolerans EJ3 produce thermo stable l-asparaginase with an optimum temperature of 85 °C (Zuo et al., 2015a).

L-asparaginases from various sources different in optimum temperature and pH for max activity. L-asparaginase from E. coli works at pH 6, the lowest pH in the list which shows that the enzyme works in a slightly acidic medium (Muneer, et al., 2020). Almost all l-asparaginases isolated and purified from microbial sources work in a basic medium that levels from a pH of 8.0 to 10. L-asparaginase obtained from Bacillus licheniformis work correctly from slightly acidic to strongly basic conditions, i.e., 6.0–10 pH . The data for the molecular mass of the enzymes show that the lowest molecular mass is for Yersinia pseudotuberculosis Q66CJ2 l-asparaginase i.e.,

36.27 kDa while the enzyme with max molecular mass of 205 ± 3 kDa on native-PAGE become produced from Pseudomonas otitidis (Mahajan et al., 2014).

Table. 2: Physiochemical properties of l-asparaginases

Strain(s)	Mass (kDa)	pH Optimum	Temp. Optimum (°C)	References
Acinetobacter baumannii (R7)	160	7.2	37	(Nsayef, 2014)
Aspergillus niger	N.R	6.5	40	(44)
Aspergillus sp	56	6.0	47	(61)
Aspergillus oryzae(CCT 3940)	115	7.0-8.0	30-40	(62)
Bacillus sp.	45	7.0-8.0	37	(63)
Bacillusaryabhattai (ITBHU02)	115	8.5	40	(64)
Bacillus megaterium (H-1)	40	7.0	40	(Zhang et al., 2015)
Bacillus firmus (AVP 18)	N.R	9.0	37	(66)
Cornyebacterium glutamicum	80	7.0	40	(67)
Cladosporium sp	121	6.3	30	(67)
Escherichia coli	153	6.0	55	(Sindhu and Manonmani, 2018)
Fusarium culmorum (ASP-87)	90	8.0	40	(Meghavarnam and Janakiraman, 2018)
Mucor hiemalis	96	7.0	37	(Monica et al., 2013)
Penicillium sp.	66	7.0	37	(Patro and Gupta, 2012)
Rhizobium etli	47	9.0	37	(Moreno et al., 2012)
Staphylococcus sp	37.5	9.0-10	37	(73)
Streptomyces gulbargensis	N.R	9.0	40	(Amena et al., 2010)
Thermus thermophiles	33	9.2	77	(Pritsa and Kyriakidis, 2001)

2. **Kinetic properties of microbial l-asparaginase**:

To recognize the rate and specificity of most of the biological processes, it is vital to know the kinetic parameters of the enzyme. Apart from understanding various properties of the enzyme during a biochemical process, kinetic parameters are important to be understood to use enzymes effectively in different commercial processes. Table 3 shows the kinetics of substrate hydrolysis of l-asparaginase for different substrates. The affinity of the substrate hydrolysis for the enzyme is calculated by Michaelis constant (Km). Vmax is defined as the maximum rate at which an enzyme is completely saturated with its substrate awareness. Some such as bacterial and fungal l-asparaginases are mentioned in Table. 3 along with the kinetic parameters. Kinetic properties depend upon the factors such as PH, temperature, type, and awareness of the substrate (Muneer et al., 2020).

Table. 3: Kinetic properties of microbial l-asparaginase

Strains	substrate	Vmax	Km (mM)	Specific activity(U/mg)	References
Streptomyces fradiae	N.R	51IU/mL	10.07	30.06	(76)
Bacillus megaterium (H-1)	l-asparagine	1.58 IU/µg	0.8	45	(Zhang et al., 2015)
Bacillus licheniformis	l-asparagine	4.03 IU	0.014	0.22	(78)
Bacillus subtilis B11—06	l-asparagine	77.51 µmol/min	0.43	9.98	(79)
Cladosporium sp.	l-asparagine	4.44 µmol/mL/min	0.100	83.3	(80)
Escherichia coli (DE3)	l-glutamine	4.032mol/min	50	1.2	(Sindhu and Manonmani, 2018)
Fusarium culmorum (ASP-87)	N.R	0.5 µmol/mL/min	3.57	3.125	(Meghavarnam and Janakiraman, 2018)
Mucor hiemalis	l-asparagine	625 U/mL	4.3	69.43	(Monica et al., 2013)
Penicillium sp.	l-asparagine	N.R	4.00	13.97	(More, 2013)
Thermococcus kodakaraensis (TK1656	l-asparagine	3300µmol/mg/min	5.5	2350	(83)
Staphylococcus sp. (OJ82)	N.R	61.4 U/mL	2.2	86	(Bacelar et al., 2017)

3. Enzyme reactions and mechanism:

The hydrolysis process occurs in two steps via the intermediate beta acyl enzyme (Fig. 1). In the first step of the process, the nucleophilic portion of the enzyme is activated by a strong base, attacking the amide carbon of the rasparagin (substrate) to produce a beta acyl enzyme intermediate. The second reaction step is to attack the ester carbon with a nucleophile activated by water molecules. (85) This mechanism is comparable to the classical mechanism of serine proteases. The activity of serine proteases depends on the group of amino acids classified as catalytic triads. This catalytic triad is composed of the nucleophilic amino acid Ser, the base histidine (His), and the acidic amino acid aspartic acid (Asp), all bound by hydrogen bonds. Lasparagnase also has the ability to catalase other reactions. For example, the rasparaginase produced by Serratia marcescens can hydrolyze 5% of glutamine compared to the hydrolysis of rasparaginase. The same effect occurs with rasparaginase produced by Escherichiacoli and Erwinia chrysanthemi. Other microorganisms such as Pseudomonas. Acinetobacter glutaminase sificans synthesizes rasparaginase, which has equal activity of asparaginase and glutaminase. In some cases, lasparaginase initiates the hydrolysis of glutamine only after the lasparaginase is completely converted to aspartic acid. In fact, glutamine is a competitive inhibitor of asparagine hydrolysis. The hydrolysis of glutamine and asparagine is similar because the structures of both amino acids are similar. Therefore, most of the microbial L-asparagine has cross-glutaminase activity, with a few exceptions such as lasparagine produced by Wolinella

succinogenes, which does not have glutaminase activity. Finally, rasparaginase can also hydrolyze aspartyl peptide amides, but the reaction yields are significantly lower. （86）

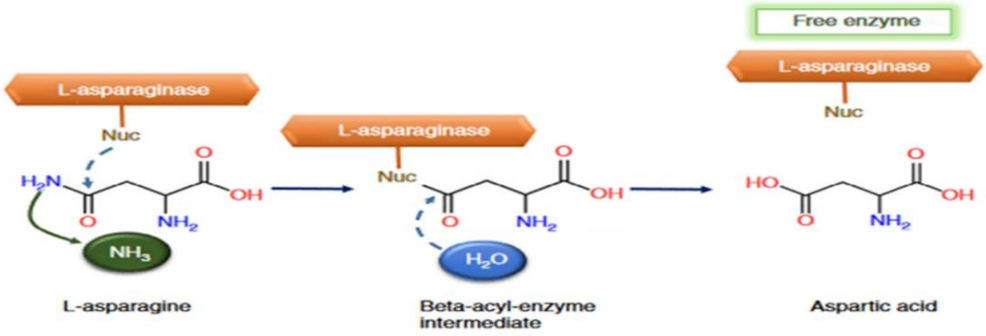

Fig1: General mechanism of l-asparaginase reaction.

4. Asparaginase Structure:

Research has been carried out by many scientists to explain the structure of L-asparaginase enzyme at the molecular level. Usually, L-asparaginase exists as a tetramer however hexameric, dimeric, and monomeric forms are also observed while isolated from different sources. Most bacterial L-asparaginases exhibit quaternary and tertiary systems. Molecular systems of E.coli and Erwinia sp. are very well investigated, and their structural data is easily available (Aghaiypour et al., 2001).

Both E. coli and Erwinia sp. have similar three-dimensional structures (Lubkowski et al., 2003). Erwinia carotovora enzyme includes tetramers (ABCD and EFGH) made up of 4 identical monomers (A to H) each. Three hundred twenty-seven amino acids assemble in each monomer with 14 α-strands, 8 β-helices (Kozak et al., 2000), and domains, a large N-terminal domain and a small C-terminal domain. The active site is placed in between adjacent monomers (A and C: B and D). (Aghaiypour et al., 2001).

The tetramer includes 4 equal subunits. The whole molecule is considered as a dimer of dimers. Every active site is shaped by the conveyance of amino acids in adjacent monomers. Following amino acids constitute (Sanches et al., 2003). Active sites: Thr15, Tyr29, Ser62, Glu63, Thr95, Asp 96, Ala120, and Lys168, while only one residue Ser254 is present in adjacent monomer. Thr15 and Thr95 are the residues responsible for the catalytic activity of the enzyme. (Asselin et al., 2000).

5. Applications of L-Asparaginase:

5.1- Applications in the healthcare industry:

Lasparaginase is a powerful anticancer drug and is widely studied in the health and pharmaceutical research community. It is registered by the World Health Organization as an essential medicine, one of the safest and most effective medicines (Muneer, et al., 2020). The antiproliferative effect of rasparaginase on leukemia cells was first studied in the 1970s. The anticancer activity of asparaginase is due to its ability to hydrolyze asparagine into aspartic acid and ammonia (87). Monitoring of asparagine clearance and anti-asparaginase antibody has been shown to be important for assessing the efficacy of rasparaginase therapy (88). Injecting or inoculating purified lasparaginase into the blood stream rapidly counters and depletes lasparagine concentrations in the blood plasma as a result of which the tumor cells are suppressed due to unavailability of asparagine, which is the highly required amino acid for the fastgrowing tumor cells E.coli derived lasparaginase is used to treat acute lymphoblastic leukemia but due to the clinical hypersensitivity these enzymes are inactivated and the desired results are not obtained(87). To avoid all these limitations, clinically modified versions of the enzyme are being used and lasparaginase from different sources is being investigated for less cytotoxic effects. Furthermore, protein engineering modifications in the purified lasparaginase has eliminated completely or partially the immunogenicity of the enzyme that has enabled it to act as an antineoplastic agent. Although there are a number of therapeutic lasparaginase products present in the market, current studies have shown that the lasparaginase from Erwinia carotovora (ERWINASE) might be more efficient with fewer side Other brands approved by the FDA include ELSPAR, KIDROLASE, and ONCASPAR which are currently being used against acute lymphoblastic leukemia and lymphosarcoma (89) . The need for new and scientifically modified versions of therapeutic enzymes is of

great interest in both biotechnology and medicine. Lasparaginase has been used for a variety of cancers, but for a variety of allergic reactions that require biobetter (a new drug developed by enhancing and improving its properties from existing peptide or protein-based biopharmacy). And because it is related to toxicity, it is not without side effects (as affinity, selectivity, stability to degradation) commercially available rasparaginase (Brumano et al., 2016).

5.2 - Application of fungal L-asparaginase in food industry:

5.2.1- Formation of acrylamide:

In 2002, the Swedish National Food Administration (SNFA) reported the presence of acrylamide in food with high carbohydrate content subjected to elevated temperatures (Mottram et al., 2002) as shown in Fig. 2. Since then, research institutions and food agencies have been investigating the toxicity, formation, mitigation, and detection of acrylamide in food. Food related to human exposure to acrylamide are potato, coffee, and bakery products (biscuits and bread) (Elshafei et al., 2012).

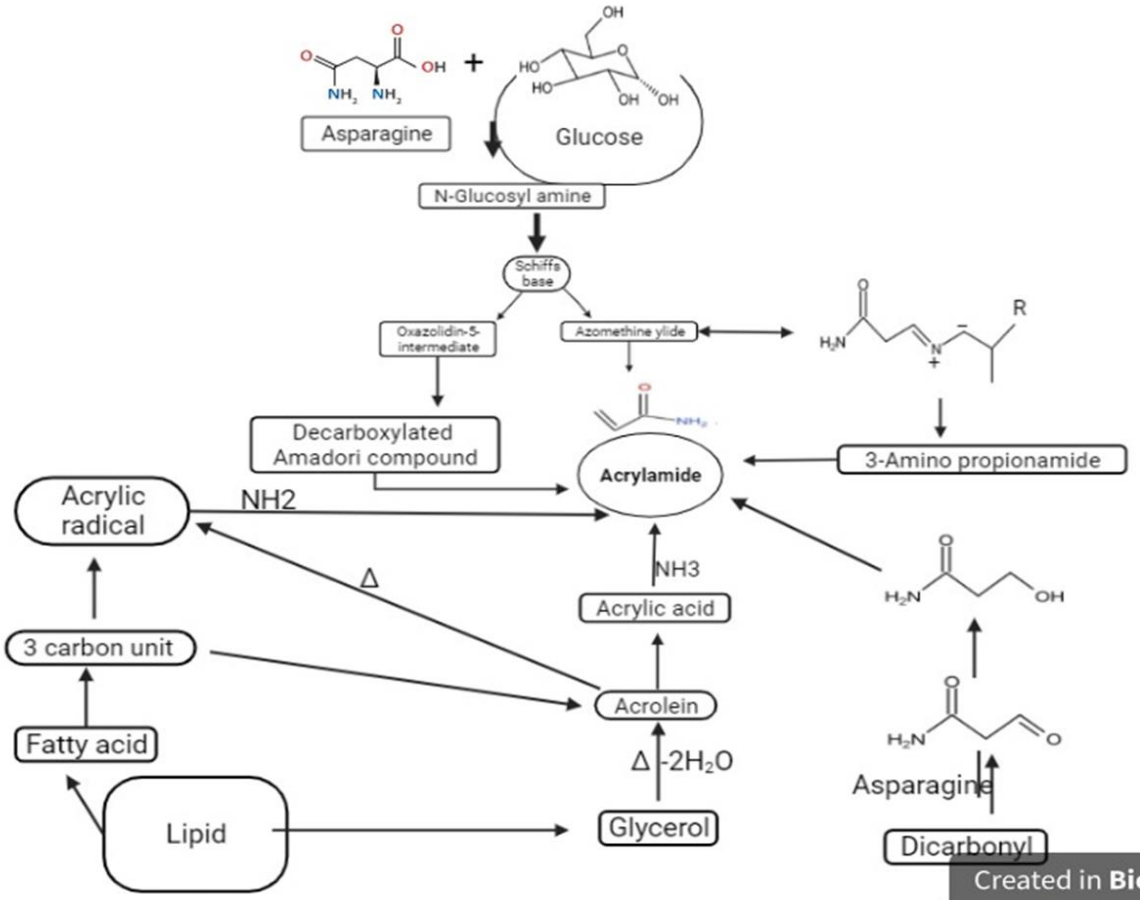

Fig. 2: Schematic diagram showing Acrylamide synthesis in foods

Estimates of food intake have been studied in several populations with different eating habits (91). When acrylamide is taken orally with food, it is estimated to be 0.3-1.9 µg / kg. However, the foods that contribute most to acrylamide intake vary from country to country depending on diet and cooking method (Claus et al., 2008). The main mechanisms accepted by researchers to explain the formation of acrylamide in foods include the Maillard reaction resulting from the reaction between food ingredients as amino acids and reducing sugars during heat treatment (92). The first step in this reaction is the formation of Schiff base intermediates. This Schiff base can then be hydrolyzed to the precursor of acrylamide, 3-aminopropionamide, or according to the figure, the amide group can be split to form acrylamide directly (Claus et al). ., 2008). 2. Several observations have led to the hypothesis that heating food may be an important cause of human exposure to acrylamide. Heating processes such as frying and baking promote the formation of acrylamide, but this compound has not been detected in cooked

foods (93). Factors that influence the formation of acrylamide in foods are processing conditions (temperature, humidity, cooking time, product matrix) and precursors such as reducing sugars and free amino acids (mainly rasparagin) (94). However, most methods of reducing acrylamide formation are aimed at removing its precursors or suppressing or reducing the intensity of the Maillard reaction through various process changes (Pedreschi et al., 2011). .. Most methods of reducing this toxic compound adversely affect both the taste and appearance of the final product (Batool et al., 2016). The use of fungal lasparaginase is a relatively new, promising and excellent technique for reducing acrylamide levels in foods. This enzyme supports mitigation strategies in two ways: it interferes with the Maillard reaction or converts rasparagine to aspartic acid (non-toxic) to remove precursors without altering the nutritional value, appearance, or taste of the final product. (Batool et al., 2016). Lasparaginase can be used as a food additive to achieve technical objectives during manufacturing. Enzymes need to be removed from food or inactivated, but the presence of trace substances or derivatives thereof is acceptable. Enzyme residues, including denatured rasparaginase, can range from 0.14 to 428 mg / kg food (Kumar and Nigam, 2012)

5.2.2- French fries:

Products derived from potatoes (Solanum tuberosum), French fries are widely consumed in many countries. Fried potato products have contributed to 50% of the human ingestion of acrylamide in European countries (Keramat et al., 2011). The amino acid Lasparagine is recognized as the main precursor for the formation of acrylamide in products derived from potatoes, so it is important to verify the performance of the enzyme in the decrease of this compound, since a high content of this amino acid in potatoes may be also related to the increase of the acrylamide formation (95). The potential of acrylamide formation in potatoes is related to several factors such as: Lasparagine content and reducing sugars, color, and moisture (in fresh potatoes), oil and utensils used for frying, content of polar compounds in the frying oil (during the frying process), color, thickness, and visual color (after frying). Other relevant factors related to the formation of acrylamide in potatoes are: potato variety, soil type, fertilization, weather, storage, cutting, blanching, and drying process, and use of additives .Though all those factors are related to acrylamide formation, reducing sugars and Lasparagine are the limiting factor for acrylamide formation in potato products (96). Acrylamide reduction in potato chips has been tested under a variety of conditions using a combination of bleach and Aspergillus oryzae lasparaginase, in addition to treatment alone. The maximum reduction (90%) was achieved when bleached (85 ° C / 3.5 minutes) and then immersed in lasparaginase solution (50 ° C / 20 minutes). Heat treatment was able to induce structural changes in potatoes, promoting the diffusion of enzymes into tissues and, as a result, promoting and enhancing their action (Pedreschi et al., 2011). A study reporting the reduction of acrylamide in french fries using the fungal lasparaginase of Aspergillus oryzae CCT 3940 (50 U / mL) showed a 72% reduction in acrylamide compared to controls (180 °). After 7 minutes frying process in C). Sample (Dias et al., 2017). Lasparaginase produced by Fusarium culmorum (ASP87) has been used to reduce acrylamide in potato products. The potato chips were enzymatically treated at 40 ° C for 30 minutes and then fried at a temperature of 170-180 ° C for 90 seconds. It was observed that 300 U / L of rasparaginase was required to reduce acrylamide levels in potato chips and french fries by 85% and 94%, respectively. Asparaginase was effective in suppressing the formation of acrylamide and reducing rasparagin. The authors also found that various types of products sourced from the local market (Bangalore, India) contained high levels of acrylamide. Values above 3020 µg / kg were observed with potato chips and 4475 µg / kg with french fries (Meghavarnam et al., 2018a).

5.2.3- Bakery products:

The color of baked goods is one of the most important attributes that influences consumer acceptability in addition to food quality (97). The Maillard reaction is involved in the formation of certain sensory properties of these products, and acrylamide is also formed during the reaction. In Europe, 20% of acrylamide intake is due to baked goods (Keramat et al., 2011). 2013 Brazilian Consumer Protection Association (Cunha et al., 2019a). Evaluating the amount of acrylamide in 51 products across eight categories: potato chips, sweet and tasty cookies, cream crackers, French bread, snack foods, and toast, French bread, candy and cookies have the highest acrylamide levels. I found that. For example, sweet biscuits show acrylamide content values of 1100 mg / kg to <100 mg> 97% by treating gingerbread with a commercially available rasparaginase enzyme without affecting the sensory quality of the final product. I did. Vass, Amrein, Schönbächler, Escher and Amado (2004) were able to reduce the acrylamide content of biscuits by up to 70% using commercially available lasparaginase. These authors also did not observe changes in color or taste. Anese, Quarta, Peloux, and Calligaris (2011) investigated the effect of biscuit composition on the ability of lasparaginase (Novozymes®) to minimize acrylamide formation. Various recipes for biscuits were tested, water composition and fat types changed, and 900 U / kg flour was added to all

treatments. The authors concluded that the difference in the efficacy of rasparaginase may be related to the different composition of the foods evaluated. Despite its ability to remove acrylamide, rasparaginase showed a better reduction of acrylamide in high water content biscuits, but the reduction of acrylamide decreased with increasing fat content in the biscuits. In another study, the effect of rasparaginase on the formation of acrylamide in biscuits was evaluated using an experimental modeling model. Anese, Quarta and Frias (2011) found that the use of medium concentrations of lasparaginase (500 U / kg) minimized acrylamide formation at the lowest incubation temperatures in the shortest time. Color, an important parameter of biscuits, was unaffected by the application of the enzyme. The rasparaginase gene from Rhizomucor miehei was modified by Huang et al. Clone and expressed in E. coli. (2014) Using an enzyme (10 U / mg flour), we reduced the acrylamide content of biscuits and achieved a reduction of about 80%. Moreover.

5.2.4- Coffee:

Along with potatoes and grain products, roasted coffee is one of the products with the highest acrylamide content. The two main types of coffee used to prepare drinks are Coflea arabica and Coflea canefora robusta. The quality of the drink depends on the proportion of each coffee bean (99). Compared to roasting Arabica coffee beans, roasting Robusta coffee beans contains higher levels of acrylamide (100). According to the results of the European Food Safety Authority (EFSA) Contaminants Panel (CONTAM) in the food chain, more than 1500 coffee-based products were analyzed between 2010 and 2013, with an average acrylamide concentration of 578 ng / roast coffee. It was g (CONTAM). , 2015). .. Coffee has high levels of acrylamide due to the roasting process. Storage conditions may also contribute to the formation of acrylamide (Mesías and Morales, 2016). Exposure to acrylamide, from roasted coffee consumption, varies from country to country, age, and sex of the consumer, roast grade and volume of coffee in gested, andsoon (100). Consequently, daily intake of this product represents a significant source of exposure to acrylamide. Another problem is related to the fact that acrylamide is a polar molecule. Preparing ground coffee with hot water can completely extract the acrylamide contained in roasted coffee beans (103). One of the precautions against the formation of acrylamide is to use lasparaginase to maintain the lowest concentration of this compound during roasting. It acts on precursors such as rasparagin, creating adverse reaction conditions (100). A study by Hendriksen, Budolfsen and Baumann (2013) evaluated the effect of asparaginase on the reduction of acrylamide in coffee. Green arabica beans were steamed in water at 100 ° C for 45 minutes and treated with rasparaginase at 60 ° C for 60 minutes. The authors observed a maximum reduction when using a concentration of 6000 ASU / kg grain, a 70-80% reduction in rasparagin content, and a 55-74% reduction in formation.

5.2.5 bread:

In the potato, wheat, and rye model systems, a linear relationship was observed in the formation of acrylamide compared to residual levels of asparagine and reducing sugars. There are many studies aimed at reducing acrylamide formation during baking: acrylamide levels follow asparagine levels and heat treatment in terms of time and temperature. Fiber bread produces higher levels of acrylamide because the highest acrylamide content is in the crust and the highest asparagine content is not in the starchy endosperm portion of the grain (104). In gingerbread, ammonium bicarbonate strongly promoted the formation of acrylamide. Both acrylamide concentration and browning intensity increase with baking time and correlate with each other. The use of baking soda as a baking agent reduced the formation of acrylamide. Reduced levels of asparagine, replacing reducing sugars with sucrose or by adding organic acids, could also lower the acrylamide content (105).

This also occurred to sweet bakeries, like biscuits. Ammonium hydrogen carbonate also affects acrylamide content in other type of bread. Ammonium hydrogen carbonate is an effective amino source in the formation of acrylamide, i.e. acrylamide content increases when ammonium hydrogen carbonate is added, but decreases when sodium hydrogen carbonate is added, which only effect the pH of the product (106).

One way of reducing free asparagine is to ferment the dough with yeast. Sourdough inhibits the asparagine utilization of the yeast (Krishnakumar et al., 2014). Added asparagine, but not fructose has increased the acrylamide content in wheat bread and rye crisp bread. In wheat bread, most of the acrylamide was in the crust (Surdyk et al., 2004; Mustafa et al., 2005). Addition of glycine reduces the content of acrylamide in cereal and potato products (Bråthen and Knutsen, 2005).

In a cracker model, sodium hydrogen carbonate eliminated acrylamide. To a lesser extent, ammonium hydrogen carbonate, cysteine, sodium bisulfite, and ascorbate also enhanced elimination. Citric acid, ferulic acid, and sodium chloride, were found to decrease the amount of acrylamide produced while having little or no effect on elimination. Asparagine, but not reducing sugar, caused a large increase in acrylamide formation (Levine and Smith, 2005).

5.2.6- Almonds:

In almonds acrylamide increases with roasting time and temperature, but temperature have much higher impact on the formation than time. During the roasting procedure sugars are consumed faster and to a larger extent than free asparagine, suggesting that the content of reducing sugars is the critical factor for formation of acrylamide in roasted almonds. Acrylamide was found to decrease in roasted almonds during storage at room temperature.(111).

Table 4: Formation of acrylamide and application of fungal L-asparaginase in food industry

Food items	Pretreatment	Reduction in acrylamide formation	Source of L-asparaginase	Reference
Cookies	Enzyme load: 500 U kg–1 pretreatment time: 11–15 Min	23-75%	Aspergillus oryzae	(112)
Gingerbread	enzyme load: 1000 U kg–1 Pretreatment time: 30–60 min,	97%	Commercial	(112)
French fries	Enzyme load: 10–20 U mL–1, pretreatment time: 5 Min	80%	Thermococcus zilligii	(113)
French fries	enzyme load: 10.5 ASNU per mL-1 Min soaking time	60-85%	Aspergillus oryzae	(Parameswaran et al.,2018)
Potato chips	300 U L–1	85%	Fusarium culmorum	(Meghavarnam et al., 2018)
Potato crisps	Enzyme load: 0–40 units	80%	Bacillus subtilis	(98)
Coffee	Enzyme load: 30 IUmL–1, various dosages	55–74%	Aspergillus oryzae	(98)
Sweet bread	Enzyme load: 50–300U, pretreatment time: 25 Min.	Crust: 97%, crumb:73%	Cladosporium sp.	(115)

Conclusion

From the past 30 years, a lot of research had been done for the role of L-asparaginase as a potent antitumour drug. It was first found that the guinea-pig serum exhibit an anti-cancer properties. Later on this drug was isolated from E.coli and Erwinia sp. which have been used in the treatment of acute lymphocytic leukemia since then. But the lower yield of the enzyme is also one of the major concerns. Not only various mode of productions and optimization conditions were adopted to isolate the enzyme from different sources all over the world to enhance its production but also a various kind of modifications of this enzyme had been tried by scientists to reduce its immunogenicity and increase its half-life. The application of L-asparaginase in the food industry for cleaving of the asparagine and elimination of cancer-causing acrylamide from baked food has been one of the eminent discoveries of mordern time.

REFERENCES

1. Offman MN, Krol M, Patel N, Krishnan S, Liu J, Saha V BP. Rational engineering of L-asparaginase reveals importance of dual activity for cancer cell toxicity. Blood. 2010;117:1614– 1621.
2. Savitri NA AW. Microbial l-asparaginase: a potent antitumour enzyme. Indian J Biotechnol. 2003;2:184–194.
3. Benchamin D, Sreejai R, Sujitha S AC. Anti-proliferative activity of l-asparaginase enzyme from fungi on breast cancer. J Pharmacogn Phytochem. 2019;8(1):407–410.
4. Cachumba JJM, Antunes FAF, Peres GFD, Brumano LP SJ, SS DS. Current applications and different approaches for microbial l-asparaginase production. Braz J Microbiol. 2016;47:77–85.
5. Ray M, Adhikari S, Kundu P. Isolation and Characterization of Microbial Asparaginase to Mitigate Acrylamide Formation in Food. Adv Plant Microb Biotechnol. 2019;13:95–100.
6. Dias FFG, Ruiz ALTG, Torre A Della, Sato HH. Purification, characterization and antiproliferative activity of l-asparaginase from Aspergillus oryzae CCT 3940 with no glutaminase activity. Asian Pac J Trop Biomed. 2016 Sep 1;6(9):785–94.
7. Shukla D, Shrivastav VK, Jana AM, Shrivastav A A EL. Exploration of the potential l-asparaginase producing bacteria from the soil of Gwalior (India). Int J Curr Microbiol App Sci. 2014;3:665–672.
8. Krishnakumar T, Visvanathan R. Citation: Krishnakumar T, Visvanathan R (2014) Acrylamide in Food Products: A Review. J Food Process Technol. 2014;5(7):344.
9. FAO/WHO. Consultation on the Health implications of acrylamide in food. FAO, Washingt. 2002;25–7.
10. Alam S, Pranaw K, Tiwari R, Khare SK. Recent Development in the Uses of Asparaginase as Food Enzyme. Energy, Environ Sustain. 2019;41:55–81.
11. Xu F, Oruna-Concha MJ, Elmore JS. The use of asparaginase to reduce acrylamide levels in cooked food. Food Chem. 2016 Nov 1;210:163–71.
12. Rodrigues D, Pillaca-Pullo O, Torres-Obreque K, Flores-Santos J, Sánchez-Moguel I, Pimenta M V., et al. Fed-batch production of Saccharomyces cerevisiae L-Asparaginase II by recombinant Pichia pastoris MUTs strain. Front Bioeng Biotechnol. 2019;7(FEB):16.
13. Narta UK, Kanwar SS, Azmi W. Pharmacological and clinical evaluation of l-asparaginase in the treatment of leukemia. Crit Rev Oncol Hematol. 2007 Mar 1;61(3):208–21.
14. Benchamin D, Sreejai R, … SS-JP, 2019 U. Anti-proliferative activity of L-Asparaginase enzyme from fungi on breast cancer. academia.edu [Internet]. 2019 [cited 2022 Jan 1];8(1):407–10. Available from: https://www.academia.edu/download/58115040/7-6-468-716.pdf
15. Batool T, Makky EA, Jalal M, Yusoff MM. A Comprehensive Review on l-Asparaginase and Its Applications. Appl Biochem Biotechnol. 2016;178(5):900–23.
16. Wriston J, Biol TY-AERAM, 1973 U. L-asparaginase: a review. books.google.com [Internet]. 1973 [cited 2021 Mar 9]; Available from: https://books.google.com/books?hl=ar&lr=&id=PFV3Y5MCPhoC&oi=fnd&pg=PA185&dq=Wriston,+J.,+%26Yellin,+T.+O.+(1973).+L-asparaginase:+a+review.+Advances+in+Enzymology+andRelated+Areas+of+Molecular+Biology,+39,185–248.&ots=hNp97leV5A&sig=1GQP20qFDxy2iIPNTvL6Ob
17. Narta U, Roy S, Kanwar S, Technology WA-B, 2011 U. Improved production of L-asparaginase by Bacillus brevis cultivated in the presence of oxygen-vectors. Elsevier [Internet]. 2011 [cited 2021 Mar 9]; Available from: https://www.sciencedirect.com/science/article/pii/S0960852410013295
18. Bascomb S, Banks GT, Skarstedt MT, Fleming A, Bettelheim KA. The properties and large scale production of L asparaginase from Citrobacter. J Gen Microbiol. 1975;91(1):1–16.
19. microbiologica JN-F, 1977 U. Stimulation of L-asparaginase production inEscherichia coli by organic and amino acids. Springer [Internet]. 1977 [cited 2021 Mar 9]; Available from: https://link.springer.com/content/pdf/10.1007/BF02881635.pdf
20. Mukherjee J, Majumdar S, Scheper T. Studies on nutritional and oxygen requirements for production of L- asparaginase by Enterobacter aerogenes. Appl Microbiol Biotechnol. 2000;53(2):180–4.
21. Nawaz MS, Zhang D, Khan AA, Cerniglia CE. Isolation and characterization of Enterobacter cloacae capable of metabolizing asparagine. Appl Microbiol Biotechnol. 1998;50(5):568–72.
22. Tiwari N, Dua RD. Purification and preliminary characterization of L-asparaginase from Erwinia aroideae NRRL B-138. Indian J Biochem Biophys [Internet]. 1996 Oct 1 [cited 2021 Mar 10];33(5):371–6. Available from: https://europepmc.org/article/med/9029817
23. Maladkar N, Singh V, Bulletin SN-HA, 1993 U. Fermentative production and isolation of L-asparaginase from Erwinia carotovora, EC-113.. europepmc.org [Internet]. 1999 [cited 2021 Mar 10]; Available from: https://europepmc.org/article/med/8181956
24. Moola ZB, Scawen MD, Atkinson T, Nicholls DJ. Erwinia chrysanthemi L-asparaginase: epitope

mapping and production of antigenically modified enzymes [Internet]. Vol. 302, Biochem. J. 1994 [cited 2021 Mar 10]. Available from: https://portlandpress.com/biochemj/article-abstract/302/3/921/31043
25. Stark RM, Suleiman M-S, Hassant IJ, Greenman J, Millari MR. Amino acid utilisation and deamination of glutamine and asparagine by Helicobacter pylori [Internet]. Vol. 46, J. Med. Microbiol. 1997 [cited 2021 Mar 10]. Available from: www.microbiologyresearch.org
26. Kotzia GA, Labrou NE. Engineering thermal stability of L-asparaginase by in vitro directed evolution. Wiley Online Libr [Internet]. 2009 Mar [cited 2021 Mar 10];276(6):1750–61. Available from: https://febs.onlinelibrary.wiley.com/doi/abs/10.1111/j.1742-4658.2009.06910.x
27. Zhang N, Clarke F, Trapani G Di, … DK-… and PPB, 1995 U. Guinea pig serum L-asparaginase: purification, and immunological relationship to liver L-asparaginase and serum L-asparaginases in other mammals. Elsevier [Internet]. 1995 [cited 2021 Mar 10]; Available from: https://www.sciencedirect.com/science/article/pii/0305049195001069
28. AIa N, Sokolov N, … EK-B (Moscow, 1975 U. Isolation and properties of a homogeneous L-asparaginase preparation from Pseudomonas fluorescens AG. europepmc.org [Internet]. 1997 [cited 2021 Mar 10]; Available from: https://europepmc.org/article/med/2329
29. Manna S, Sinha A, Sadhukhan R, Microbiology SC-C, 1995 U. Purification, characterization and antitumor activity of L-asparaginase isolated from Pseudomonas stutzeri MB-405. Springer [Internet]. 1995 [cited 2021 Mar 10]; Available from: https://link.springer.com/article/10.1007/BF00295504
30. Yao M, Yasutake Y, Morita H, … IT-CSD, 2005 U. Structure of the type I L-asparaginase from the hyperthermophilic archaeon Pyrococcus horikoshii at 2.16 Å resolution. scripts.iucr.org [Internet]. 2005 [cited 2021 Mar 10]; Available from: https://scripts.iucr.org/cgi-bin/paper?hv5026
31. Pritsa AA, Kyriakidis DA. L-asparaginase of Thermus thermophilus: Purification, properties and identification of essential amino acids for its catalytic activity [Internet]. Vol. 216, Molecular and Cellular Biochemistry. 2001 [cited 2021 Mar 10]. Available from: https://idp.springer.com/authorize/casa?redirect_uri=https://link.springer.com/article/10.1023/A:1011066129771&casa_token=aJROXwHWxRwAAAAA:FThsGEoXHAqPx7z9yW1DSOUw7-7W-LjMM3PgsuZhIuY-YgFdz2VHQE2EAq-lSF3Ic4k88zyOirV8Ay-v9Q
32. Curran M, Daniel R, Guy G, Biophysics HM and, 1985 U. A specific L-asparaginase from Thermus aquaticus. Elsevier [Internet]. 1985 [cited 2021 Mar 10]; Available from: https://www.sciencedirect.com/science/article/pii/000398618590582X
33. Distasio J, Niederman R, Chemistry DK-… of B, 1976 U. Purification and characterization of L-asparaginase with anti-lymphoma activity from Vibrio succinogenes.. Elsevier [Internet]. 1976 [cited 2021 Mar 10]; Available from: https://www.sciencedirect.com/science/article/pii/S0021925817329241
34. Tosa T, Sano R, Yamamoto K, Nakamura M, Chibata I. L-Asparaginase from Proteus vulgaris. Purification, Crystallization, and Enzymic Properties. Biochemistry. 1972 Jan 1;11(2):217–22.
35. Prakasham RS, Hymavathi M, Subba Rao C, Arepalli SK, Venkateswara Rao J, Kennady PK, et al. Evaluation of antineoplastic activity of extracellular asparaginase produced by isolated bacillus circulans. Appl Biochem Biotechnol. 2010 Jan;160(1):72–80.
36. Batool T, Makky EA, Jalal M, Yusoff MM. A Comprehensive Review on L-Asparaginase and Its Applications. Springer [Internet]. 1971 [cited 2021 Mar 10]; Available from: https://idp.springer.com/authorize/casa?redirect_uri=https://link.springer.com/content/pdf/10.1007/s12010-015-1917-3.pdf&casa_token=gHnEqBBT0usAAAAA:lRNdwENmr0BeXxZPCtjxoxGd5TzQcriYaYDTyRVsQZ3vYEd0PbSA7jl_YNBW1DD29V825HLu3S4_5hxhDQ
37. Mohapatra BR, Sani RK, Banerjee UC. Characterization of L-asparaginase from Bacillus sp. isolated from an intertidal marine alga (Sargassum sp.). Lett Appl Microbiol. 1995;21(6):380–3.
38. Nefelova M, Ignatov S, … AC-P, 1978 U. Biosynthesis of L-asparaginase-2 by cultures of Bacillus polymyxa var. Ross. europepmc.org [Internet]. 2006 [cited 2021 Mar 10]; Available from: https://europepmc.org/article/med/724659
39. Fisher SH, Wray L V. Bacillus subtilis 168 Contains Two Differentially Regulated Genes Encoding L-Asparaginase. J Bacteriol [Internet]. 2002 [cited 2021 Mar 10];184(8):2148–54. Available from: http://jb.asm.org/
40. Hymavathi M, Sathish T, Rao CS, Prakasham RS. Enhancement of l-asparaginase production by isolated bacillus circulans (MTCC 8574) using response surface methodology. Appl Biochem Biotechnol. 2009 Oct;159(1):191–8.
41. Mesas JM, Gil JA, Martin JF. Characterization and partial purification of L-asparaginase from Corynebacterium glutamicum. J Gen Microbiol. 1990;136(3):515–9.

42. Sobiś M, Polonica JM-AM, 1991 U. Staphylococcal L-asparaginase: enzyme kinetics.. europepmc.org [Internet]. 1991 [cited 2021 Mar 10]; Available from: https://europepmc.org/article/med/1726615
43. Kil J, Kim G, - IP, biotechnology undefined, biochemistry and, 1995 undefined. Bioscience, Biotechnology, and Biochemistry Extraction of Extracellular L-Asparaginase from Candida utilis. Taylor Fr [Internet]. 2014 [cited 2021 Mar 10];59(4):749–50. Available from: https://www.tandfonline.com/action/journalInformation?journalCode=tbbb20
44. Mishra A. Production of L-asparaginase, an anticancer agent, from Aspergillus niger using agricultural waste in solid state fermentation. Appl Biochem Biotechnol. 2006 Oct;135(1):33–42.
45. Daverey A, Pakshirajan K. Kinetics of growth and enhanced sophorolipids production by candida bombicola using a low-cost fermentative medium. Appl Biochem Biotechnol. 2010 Apr;160(7):2090–101.
46. Nagarajan A, … NT-RJ of, 2014 U. Screening and isolation of novel glutaminase free L-asparaginase from fungal endophytes. search.proquest.com [Internet]. 2014 [cited 2021 Mar 10]; Available from: https://search.proquest.com/openview/57004102af36e343f47172d416e5d17c/1.pdf?pq-origsite=gscholar&cbl=276275
47. SAXENA R. L-asparaginase and glutaminase activities in the culture filtrates of Aspergillus nidulans . 1981 [Internet]. 1981 [cited 2021 Mar 10]; Available from: https://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=PASCALAGROLINEINRA8110345791
48. Sarquis M, Oliveira E, Cruz AS-… IO, 2004 U. Production of L-asparaginase by filamentous fungi. SciELO Bras [Internet]. 2004 [cited 2021 Mar 10]; Available from: https://www.scielo.br/scielo.php?pid=S0074-02762004000500005&script=sci_arttext&tlng=pt
49. Hendriksen H V., Kornbrust BA, Ostergaard 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 May 27;57(10):4168–76.
50. Raha S, Roy S, … SD-B, 1990 U. Purification and properties of an L-asparaginase from Cylindrocarpon obtusisporum MB-10.. europepmc.org [Internet]. 1990 [cited 2021 Mar 10]; Available from: https://europepmc.org/article/med/2080924
51. Mohapatra BR, Bapuji M. Characterization of urethanase from Micrococcus species associated with the marine sponge (Spirastrella species). Lett Appl Microbiol. 1997;25(6):393–6.
52. Nakahama K, Imada A, Igarasi S, Tubaki K. Formation of L-asparaginase by fusarium species. J Gen Microbiol. 1973;75(2):269–73.
53. Narayana KJP, Kumar · K G, Vijayalakshmi · M. L-asparaginase production by Streptomyces albidofl avus. Indian J Microbiol [Internet]. 2008 [cited 2021 Mar 10];48:331–6. Available from: https://idp.springer.com/authorize/casa?redirect_uri=https://link.springer.com/content/pdf/10.1007/s12088-008-0018-1.pdf&casa_token=p-5LwY9er1YAAAAA:aQTEfi61b53YyCOBeNmvV3fA_QbtfLLoVP2XlgNi0bJG1M0nPX8u5WXObyjX5qcvxO2zs-Fa4SZ_OctL-SE
54. Gupta N, Mishra S, Basak UC. Occurrence of Streptomyces aurantiacus in mangroves of Bhitarkanika. Malays J Microbiol [Internet]. 2007 [cited 2021 Mar 10];3(2):7–14. Available from: http://dx.doi.org/10.21161/mjm.00807
55. Mostafa S, Landwirtschaft MS- der, Des der T und, 1979 U. L-asparaginase-producing Streptomyces from the soil of Kuwait. Elsevier [Internet]. 1979 [cited 2021 Mar 10]; Available from: https://www.sciencedirect.com/science/article/pii/S0323605679800051
56. Dejong PJ. L-Asparaginase Production by Streptomyces griseus Downloaded from [Internet]. APPLiD MICROBIOLOGY. 1972 [cited 2021 Mar 10]. Available from: http://aem.asm.org/
57. Amena S, Vishalakshi N, Prabhakar M, Dayanand A, Lingappa K. Production, purification and characterization of L-asparaginase from Streptomyces gulbargensis. Brazilian J Microbiol [Internet]. 2010 [cited 2021 Mar 10];41(1):173–8. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1517-83822010000100025&lng=en&nrm=iso&tlng=en
58. Landwirtschaft SM-M der, Und der T, 1979 U. Activity of L-asparaginase in cells of Streptomyces karnatakensis. Elsevier [Internet]. 1979 [cited 2021 Mar 10]; Available from: https://www.sciencedirect.com/science/article/pii/S0323605679800075
59. Abdel-Fattah Y, Biochemistry ZO-P, 2002 U. L-asparaginase production by Pseudomonas aeruginosa in solid-state culture: evaluation and optimization of culture conditions using factorial designs. Elsevier [Internet]. 2002 [cited 2021 Mar 10]; Available from: https://www.sciencedirect.com/science/article/pii/S0032959202000675

60. Nsayef Muslim Ȧ S. Production, Purification and Characterization of a novel L-asparaginase from Acinetobacter baumannii with anticancerous activity [Internet]. Vol. 4, Research Article International Journal of Current Engineering and Technology. 2014 [cited 2021 Mar 14]. Available from: http://inpressco.com/category/ijcet
61. Qeshmi F, Homaei A, Research PF-M, 2018 U. Marine microbial L-asparaginase: biochemistry, molecular approaches and applications in tumor therapy and in food industry. Elsevier [Internet]. 2018 [cited 2021 Mar 15]; Available from: https://www.sciencedirect.com/science/article/pii/S0944501317312673
62. Depeursinge A, Racoceanu D, Iavindrasana J, Cohen G, Platon A, Poletti P-A, et al. Fusing Visual and Clinical Information for Lung Tissue Classification in HRCT Data. Artif Intell Med. 2010;ARTMED1118.
63. Alagarsamy S, Moorthy V, Ramalingam A, Sumantha A, Tippapur Shankaranaya R. Production, purification and characterisation of extracellular L-asparaginase from a soil isolate of Bacillus sp. Artic African J Microbiol Res [Internet]. 2010 [cited 2021 Mar 15];4(18):1862–7. Available from: https://www.researchgate.net/publication/228616101
64. Singh Y, Gundampati RK, Jagannadham M V., Srivastava SK. Extracellular l-asparaginase from a protease-deficient bacillus aryabhattai ITBHU02: Purification, biochemical characterization, and evaluation of antineoplastic activity in vitro. Appl Biochem Biotechnol. 2013 Dec;171(7):1759–74.
65. Zhang S, Xie Y, Zhang C, Bie X, Zhao H, … FL-FR, et al. Biochemical characterization of a novel l-asparaginase from Bacillus megaterium H-1 and its application in French fries. Elsevier [Internet]. 2015 [cited 2021 Mar 15]; Available from: https://www.sciencedirect.com/science/article/pii/S0963996915301654
66. Muneer F, Muhammad ·, Siddique H, Azeem · Farrukh, Rasul I, Muzammil S, et al. MINI-REVIEW Microbial l-asparaginase: purification, characterization and applications. Springer [Internet]. 2020 [cited 2021 Mar 15];202:967–81. Available from: https://doi.org/10.1007/s00203-020-01814-1
67. Kumar K, Kataria M, Verma N. Artificial Cells, Nanomedicine, and Biotechnology Plant asparaginase-based asparagine biosensor for leukemia Plant asparaginase-based asparagine biosensor for leukemia. Nanomedicine, Biotechnol [Internet]. 2012 [cited 2021 Mar 16];41(3):184–8. Available from: https://www.tandfonline.com/action/journalInformation?journalCode=ianb20
68. Sindhu R, Manonmani HK. Expression and characterization of recombinant L-asparaginase from Pseudomonas fluorescens. Protein Expr Purif. 2018 Mar 1;143:83–91.
69. Meghavarnam AK, Janakiraman S. Evaluation of acrylamide reduction potential of L-asparaginase from Fusarium culmorum (ASP-87) in starchy products. LWT. 2018 Mar 1;89:32–7.
70. Monica T, Lincoln L, Niyonzima F, of SS-, 2013 undefined. Isolation, purification and characterization of fungal extracellular L-asparaginase from Mucor hiemalis. researchgate.net [Internet]. 2013 [cited 2021 Mar 16]; Available from: http://dx.doi.org/10.4172/2324-9099.1000108
71. Patro KR, Gupta N. Extraction, purification and characterization of L-asparaginase from Penicillium sp. by submerged fermentation. Int J Biotechnol Mol Biol Res [Internet]. 2012 [cited 2021 Mar 16];3(3):30–4. Available from: http://www.academicjournals.org/IJBMBR
72. Moreno-Enríquez A, Evangelista-Martínez Z, González-Mondragón EG, Calderón-Flores A, Arreguín R, Pérez-Rueda E, et al. Biochemical Characterization of Recombinant L-Asparaginase (AnsA) from Rhizobium etli, a Member of an Increasing Rhizobial-Type Family of L-Asparaginases. J Microbiol Biotechnol [Internet]. 2012 [cited 2021 Mar 16];22(3):292–300. Available from: http://dx.doi.org/10.4014/jmb.1107.07047http://jmb.or.kr.
73. Han S, Jung J, Park W. Biochemical Characterization of L-Asparaginase in NaCl-Tolerant Staphylococcus sp. OJ82 Isolated from Fermented Seafood. J Microbiol Biotechnol [Internet]. 2014 May 27 [cited 2021 Mar 16];24(8):1096–104. Available from: http://dx.doi.org/10.4014/jmb.1405.05021
74. Amena S, Vishalakshi N, … MP-B journal of, 2010 U. Production, purification and characterization of L-asparaginase from Streptomyces gulbargensis. SciELO Bras [Internet]. 2010 [cited 2021 Mar 16]; Available from: https://www.scielo.br/scielo.php?pid=S1517-83822010000100025&script=sci_arttext
75. Pritsa AA, Kyriakidis DA. L-asparaginase of Thermus thermophilus: Purification, properties and identification of essential amino acids for its catalytic activity. Mol Cell Biochem. 2001;216(1–2):93–101.
76. El-Naggar NEA, Deraz SF, Soliman HM, El-Deeb NM, El-Ewasy SM. Purification, characterization, cytotoxicity and anticancer activities of L-asparaginase, anti-colon cancer protein, from the newly isolated alkaliphilic Streptomyces fradiae NEAE-82. Sci Rep [Internet]. 2016 Sep 8 [cited 2021 Mar 11];6(1):1–16. Available from: www.nature.com/scientificreports

77. Zhang S, Xie Y, Zhang C, Bie X, Zhao H, … FL-FR, et al. Biochemical characterization of a novel l-asparaginase from Bacillus megaterium H-1 and its application in French fries. Elsevier [Internet]. 2015 [cited 2021 Mar 11]; Available from: https://www.sciencedirect.com/science/article/pii/S0963996915301654
78. Mahajan R V., Kumar V, Rajendran V, Saran S, Ghosh PC, Saxena RK. Purification and characterization of a novel and robust L-asparaginase having low-glutaminase activity from bacillus licheniformis: In vitro evaluation of anti-cancerous properties. PLoS One. 2014 Jun 6;9(6).
79. Jia M, Xu M, He B, Rao Z. Cloning, expression, and characterization of L-asparaginase from a newly isolated Bacillus subtilis B11-06. J Agric Food Chem. 2013 Oct 2;61(39):9428–34.
80. Kumar K, Kataria M, Verma N. Plant asparaginase-based asparagine biosensor for leukemia. Artif Cells, Nanomedicine, Biotechnol [Internet]. 2013 Jun 19 [cited 2021 Mar 11];41(3):184–8. Available from: http://www.tandfonline.com/doi/full/10.3109/10731199.2012.716062
81. Monica T, Lincoln L, Niyonzima F, of SS-, 2013 undefined. Isolation, purification and characterization of fungal extracellular L-asparaginase from Mucor hiemalis. researchgate.net [Internet]. 2013 [cited 2021 Mar 11]; Available from: http://dx.doi.org/10.4172/2324-9099.1000108
82. S More S. Isolation, Purification and Characterization of Fungal Extracellular L-Asparaginase from Mucor hiemalis. J Biocatal Biotransformation. 2013 Dec 21;02(02).
83. Chohan S, Bioengineering NR-J of bioscience and, 2013 U. TK1656, a thermostable L-asparaginase from Thermococcus kodakaraensis, exhibiting highest ever reported enzyme activity. Elsevier [Internet]. 2013 [cited 2021 Mar 11]; Available from: https://www.sciencedirect.com/science/article/pii/S1389172313001254
84. Bacelar Gonçalves A, Carolina Ferreira Maia A, Andres Rueda J, Paula de Figueiredo Conte Vanzela A. Fungal production of the anti-leukemic enzyme L-asparaginase: from screening to medium development. Acta Sci Biol Sci Mar [Internet]. 2017 [cited 2021 Mar 11];38:387–94. Available from: http://www.uem.br/acta
85. Lubkowski J, Vanegas J, Chan W-K, Lorenzi PL, Weinstein JN, Sukharev S, et al. Mechanism of catalysis by L-asparaginase. Biochemistry. 2020;59(20):1927–45.
86. Noronkoski T, Stoineva IB, Ivanov IP, Petkov DD, Mononen I. Glycosylasparaginase-catalyzed Synthesis and Hydrolysis of β-Aspartyl Peptides. J Biol Chem [Internet]. 1998 Oct 9 [cited 2021 Mar 30];273(41):26295–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0021925819597472
87. Krishnapura PR, Belur PD, Subramanya S. A critical review on properties and applications of microbial l-asparaginases. Crit Rev Microbiol. 2016 Sep 2;42(5):720–37.
88. Paillassa J, Leguay T, Thomas X, Huguet F, Audrain M, Lheritier V, et al. Monitoring of asparagine depletion and anti-l-asparaginase antibodies in adult acute lymphoblastic leukemia treated in the pediatric-inspired GRAALL-2005 trial [Internet]. Vol. 8, Blood Cancer Journal. Nature Publishing Group; 2018 [cited 2021 Apr 4]. p. 45. Available from: https://doi.org/
89. Mohamed SA, Elshal MF, Kumosani TA, Aldahlawi AM, Basbrain TA, Alshehri FA, et al. L-Asparaginase Isolated from Phaseolus vulgaris Seeds Exhibited Potent Anti-Acute Lymphoblastic Leukemia Effects In-Vitro and Low Immunogenic Properties In-Vivo. Int J Environ Res Public Heal 2016, Vol 13, Page 1008 [Internet]. 2016 Oct 14 [cited 2022 Jan 13];13(10):1008. Available from: https://www.mdpi.com/1660-4601/13/10/1008/htm
90. Mottram, D. S., Wedzicha, B. L., & Dodson A. Acrylamide is formed in the Maillard reaction. Nature. 2002;419(6906):448–9.
91. Dybing E, Farmer PB, Andersen M, Fennell TR, Lalljie SPD, Müller DJG, et al. Human exposure and internal dose assessments of acrylamide in food. Food Chem Toxicol. 2005 Mar 1;43(3):365–410.
92. da Cunha MC, dos Santos Aguilar JG, de Melo RR, Nagamatsu ST, Ali F, de Castro RJS, et al. Fungal L-asparaginase: Strategies for production and food applications. Food Res Int. 2019;126:108658.
93. Keramat J, LeBail A, Prost C, Jafari M. Acrylamide in Baking Products: A Review Article [Internet]. Vol. 4, Food and Bioprocess Technology. Springer; 2011 [cited 2021 Apr 15]. p. 530–43. Available from: https://link.springer.com/article/10.1007/s11947-010-0495-1
94. Zuo S, Zhang T, Jiang B, Mu W. Recent research progress on microbial l-asparaginases [Internet]. Vol. 99, Applied Microbiology and Biotechnology. Springer Verlag; 2015 [cited 2021 Apr 15]. p. 1069–79. Available from: https://link.springer.com/article/10.1007/s00253-014-6271-9
95. 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 Jun 16;52(12):3801–6.
96. Muttucumaru N, Powers SJ, Elmore JS, Dodson A, Briddon A, Mottram DS, et al. Acrylamide-forming potential of potatoes grown at different locations, and the ratio of free asparagine to reducing sugars at

which free asparagine becomes a limiting factor for acrylamide formation. Food Chem. 2017;220:76–86.
97. Bartkiene E, Jakobsone I, … IP-L-FS and, 2016 U. Reducing of acrylamide formation in wheat biscuits supplemented with flaxseed and lupine. Elsevier [Internet]. 2016 [cited 2021 Apr 15];65:275–82. Available from: https://www.sciencedirect.com/science/article/pii/S0023643815300967
98. Onishi Y, Prihanto AA, Yano S, Takagi K, Umekawa M, Wakayama M. Effective treatment for suppression of acrylamide formation in fried potato chips using L-asparaginase from Bacillus subtilis. 3 Biotech. 2015 Oct 22;5(5):783–9.
99. Mesias M, Delgado-Andrade C, Holgado F, Morales FJ. Acrylamide content in French fries prepared in households: A pilot study in Spanish homes. Food Chem. 2018;260:44–52.
100. Silva SS Da. Current applications and different approaches for microbial l -asparaginase production. Brazilian J Microbiol [Internet]. 2016;1–9. Available from: http://dx.doi.org/10.1016/j.bjm.2016.10.004
101. (CONTAM) EP on C in the FC. Scientific opinion on acrylamide in food. Efsa J. 2015;13(6):4104.
102. Mesías M, Morales FJ. Acrylamide in coffee: Estimation of exposure from vending machines. J Food Compos Anal. 2016;48:8–12.
103. Guenther PM, Reedy J, Krebs-Smith SM, Reeve BB, Basiotis PP. Development and evaluation of the healthy eating index-2005. United States. Department of Agriculture. Center for Nutrition Policy and …; 2007.
104. Elmore JS, Koutsidis G, Dodson AT, Mottram DS, Wedzicha BL. Measurement of acrylamide and its precursors in potato, wheat, and rye model systems. J Agric Food Chem. 2005;53(4):1286–93.
105. 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.
106. Haase NU, Grothe K-H, Matthäus B, Vosmann K, Lindhauer MG. Acrylamide formation and antioxidant level in biscuits related to recipe and baking. Food Addit Contam Part A. 2012;29(8):1230–8.
107. Surdyk N, Rosén J, Andersson R, Åman P. Effects of asparagine, fructose, and baking conditions on acrylamide content in yeast-leavened wheat bread. J Agric Food Chem. 2004;52(7):2047–51.
108. Mustafa A, Andersson R, Rosén J, Kamal-Eldin A, Åman P. Factors influencing acrylamide content and color in rye crisp bread. J Agric Food Chem. 2005;53(15):5985–9.
109. Bråthen E, Knutsen SH. Effect of temperature and time on the formation of acrylamide in starch-based and cereal model systems, flat breads and bread. Food Chem. 2005;92(4):693–700.
110. Levine RA, Smith RE. Sources of variability of acrylamide levels in a cracker model. J Agric Food Chem. 2005;53(11):4410–6.
111. Amrein TM, Lukac H, Andres L, Perren R, Escher F, Amadò R. Acrylamide in roasted almonds and hazelnuts. J Agric Food Chem. 2005;53(20):7819–25.
112. Parameswaran B, Varjani S, Raveendran S. Green bio-processes: enzymes in industrial food processing . 2018 [Internet]. 2018 [cited 2021 Mar 10]; Available from: https://books.google.com/books?hl=ar&lr=&id=2Q92DwAAQBAJ&oi=fnd&pg=PR5&dq=Alam,+S.,+Pranaw,+K.,+Tiwari,+R.,+and+Khare,+S.+K.+(2019)+In+Green+Bio-+processes,+Enzymes+in+Industrial+Food+Processing (Parameswaran,+B.,+Varjani,+S.,+an
113. Zuo S, Zhang T, Jiang B, Mu W. Reduction of acrylamide level through blanching with treatment by an extremely thermostable l-asparaginase during French fries processing. Extremophiles. 2015 Jul 24;19(4):841–51.
114. Meghavarnam A, LWT SJ-, 2018 U. Evaluation of acrylamide reduction potential of l-asparaginase from Fusarium culmorum (ASP-87) in starchy products. Elsevier [Internet]. 2018 [cited 2021 Mar 11]; Available from: https://www.sciencedirect.com/science/article/pii/S0023643817307235
115. Xu F, Oruna-Concha M, Chemistry JE-F, 2016 U. The use of asparaginase to reduce acrylamide levels in cooked food. Elsevier [Internet]. 2014 [cited 2021 Mar 11]; Available from: http://centaur.reading.ac.uk/64518/www.reading.ac.uk/centaur