SOURCES AND APPLICATIONS OF L-ASPARAGINASE: A REVIEW

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ABSTRACT:*L-Asparaginase, an enzyme with multifaceted applications, has garnered significant attention across various fields. In cancer therapy, L-asparaginase stands as a cornerstone treatment for leukemia, specifically acute lymphoblastic leukemia (ALL), by depriving cancer cells of the amino acid asparagine essential for their growth. In the realm of biotechnology, L-asparaginase emerges as a vital tool for the production of recombinant proteins. Mainly this enzymes is used in food industry to reduce the acrylamide formation, acrylamide is a carcinogen compound. In agriculture, L-asparaginase holds promise as a means to enhance nitrogen utilization in plants. These characteristics may result in higher crop yields, better-quality crops, and a decreased need for artificial nitrogen fertilizers.*

KEYWORDS: L-Asparaginase, Lymphoblastic leukemia, Recombinant proteins, Carcinogen compound, Nitrogen fertilizer.

INTRODUCTION:

Enzymes are biological molecules that function in living things as catalysts, accelerating and facilitating a range of metabolic activities. They play a fundamental role in the functioning of cells and are essential for life. Proteins or molecules of RNA are known as enzymes because they speed up chemical reactions without being consumed. They enable vital cellular processes to occur at the appropriate rates, allowing organisms to maintain life-sustaining activities. Enzymes are central to metabolic pathways, which are interconnected sequences of enzymatic reactions that process nutrients, produce energy, and synthesize essential molecules. The enzymes are necessary for metabolic reactions to proceed quickly enough to support life. Enzyme activity can be regulated to control cellular processes. This regulation can be achieved through factors like pH, temperature, and the presence of regulatory molecules. Enzyme activity can also be controlled through processes like allosteric regulation and feedback inhibition(1).

The distribution of L-asparaginase in plants, animals, and microbes is widespread. Because they are simpler to cultivate and easier to extract and purify, microorganisms provide a better source of L-asparaginase, allowing for more efficient large-scale manufacturing. Lymphoblastic leukemia is treated with L-asparaginase. Tetrameric protein, this molecule has a molecular weight of above 140,000 (2). To manufacture L-asparaginase, *Erwinia caratovora*, *Bacillus spp*., and *Corynebacterium glutamicum* are often used microorganisms. *Escherichia coli* with *Pseudomonas stutzeri*. The enzyme L-asparaginase from E. coli inhibits tumour growth. Additionally, *E. chrysanthemi* has pharmacological activity (3).L-asparaginase has drawn attention recently because of its important role in the food sector, where it is used to stop the development of acrylamide when foods are cooked at high temperatures. There are various commercial and pharmacological uses for various asparaginase varieties(4).Asparaginase is most commonly used as a food processing aid to reduce the formation of acrylamide, a substance that may cause cancer when used in starchy food products like biscuits and snacks. The main process that produces acrylamide when food is prepared involves the interaction of asparagine and reducing sugar at temperatures higher than 120˚C and low moisture content. The Maillard reaction, which also gives baked, roasted, and fried foods their flavor, aroma, and color, is primarily responsible for the production of acrylamide in food. (5).

SOURCE OF L-ASPARAGINASE:

L-asparaginases are found in a variety of bacterial species as well as in birds, animals, plants, fungi, and actinomycetes. There are several sources for this enzyme, however due to its enormous diversity, rapid reproduction rate, and superior economics, microbial sources are favoured over others. The production of this enzyme from various microorganisms depends primarily on the microorganism strain and fermentation conditions(6).

BACTERIAL SOURCES:

Several bacterial species can manufacture it, but the type-II enzymes from *E. coli* and *Erwinia* are synthesized on an industrial scale for clinical use. Although the medications from the two sources have the same modes of action and toxicities, their pharmacokinetic characteristics are dissimilar, and patients who are allergic to one source of the enzyme are frequently resistant to the other. In particular for therapeutic reasons in the treatment of cancer, E. coli is one of the most often used bacterial sources for the manufacture of L-asparaginase. Additionally, L-asparagine substrate is more amenable to the *E. coli* enzyme than the *E. Chrysanthemi* enzyme. Except for a few number that are released outside the cell, the majority of microbial L-asparaginase is intracellular in nature. Because gram-positive bacteria lack a periplasmic area, they secrete many enzymes into the surrounding media that gram-negative bacteria would normally have in their periplasm. This suggests that it would be better to screen gram positive bacteria in order to obtain extracellular enzyme(6,7).

FUNGAL SOURCES:

Aspergillus niger is a fungus known for its ability to produce L-asparaginase. This fungal-derived enzyme is primarily used in industrial applications, particularly in the food industry. The main aim is to decrease the production of acrylamide, a substance that may cause cancer, when food is cooked, such as when it is fried or baked. Asparagine is one of the amino acids that reacts with sugars at high temperatures to form acrylamide. By breaking down asparagine, L-asparaginase increases food safety by lowering the formation of acrylamide. L-asparaginase produced from *Aspergillus niger* is subject to regulatory approval and safety assessments in the food industry, just like any other food additive. (7,8).

PLANT SOURCES AND OTHER SOURCES:

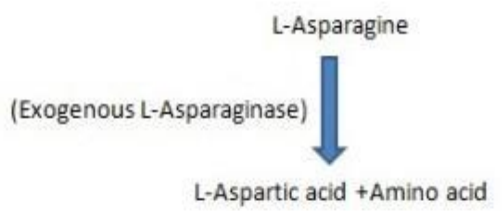
Asparagine is a key molecule for the storage and transportation of nitrogen in plants. It accumulates during physiological events like seed germination and in response to stressful situations like pathogen invasion, mineral shortage, and drought. For the growth and development of plants, L-asparaginase releases ammonia from the asparagine that has been stored. Numerous plant sources, including *Tamarindus indica*, *Capsicum annum*, *Withania somnifera*, *Vicia faba*, *Lupinus angustifolius*, and *Phaseoulus vulgaris*, have been identified in the literature as L-asparaginase producers(9).

ASPARAGINE AMINO ACID:

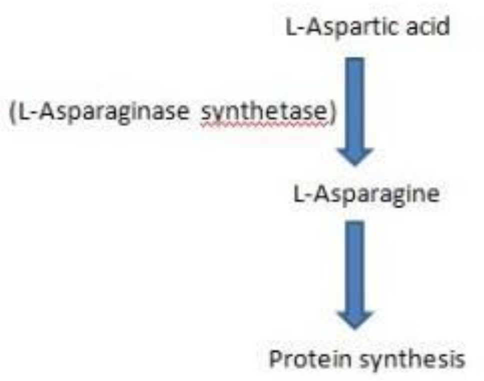
A non-essential amino acid called asparagine is crucial for cell development and proliferation. An enzyme called L-asparaginase is used to treat paediatric acute lymphoblastic leukaemia (ALL), and it has helped to raise survival rates to 90%. Because leukemic blast cells cannot manufacture enough asparagine on their own to support proliferation, they must obtain it from outside sources. This is why L-asparaginase functions as an antileukemic agent(10). L-asparaginase therapy can stop leukemic cells from progressing through their cell cycles, starving them to death if the amount of asparagine in the blood is reduced to 3 uM or less. The treatment has no effect on healthy cells that are able to produce asparagine on their own by using the enzyme asparagine synthetase (ASNS), which catalyzes the conversion of aspartate to asparagine using glutamine as a source. (11).

MECHANISM OF ACTION :

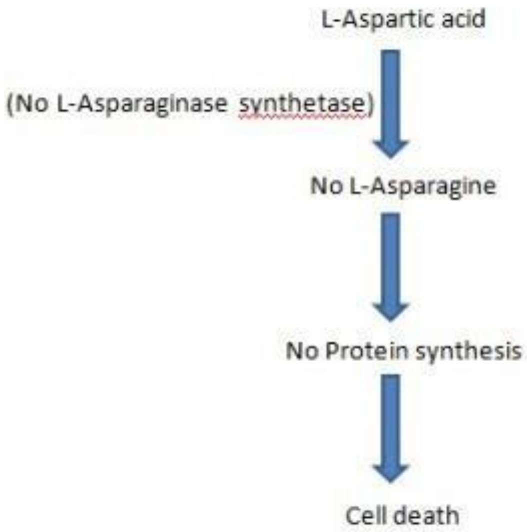
Huge amounts of asparagine are needed by tumor cells to maintain their rapid, malignant growth. This means that in order to meet their high demand for L-asparagine, they use both asparagine from blood serum and asparagine that they can make themselves. Tumor cells have an enormous requirement for the amino acid asparagine, which is exploited by the drug L-asparaginase. The hydrolysis of L-asparagine into L-aspartic acid and ammonia is catalyzed by L-asparaginase. It occurs in the bloodstream.(11,12).
L-Asparagine is converted into L-Asparatic acid and ammonia by using the enzyme L-Asparaginase(12).



It is takes place in normal cells. Where L-Aspartic acid is converted into L-Asparagine by using the L-Asparagine synthetase. Then, to protein synthesis(12).



Asparaginase is an essential amino acid required for protein synthesis. Cancer cells need it for their rapid growth and division. By depleting L-Asparagine, L-Asparaginase deprives cancer cells of this essential building block. As a result, cancer cells are unable to synthesis proteins effectively, leading to impaired growth and ultimately, cell death(12,13).



L-ASPARAGINASE ROLE IN THE FOOD INDUSTRY:

Asparaginase is a food processing enhancer that helps starchy food products produce less acrylamide, which has been linked to cancer. When meals containing carbohydrates are cooked beyond 120°C, a chemical molecule called acrylamide is created from L-asparagine and reducing sugar(14,15). Breads and other baked products, fried or baked potatoes, and reaction flavors are a few examples of these foods.The Maillard reaction is the process by which asparagine, an amino acid that is naturally present in starchy foods, is heated and turns into acrylamide(14).When asparaginase is added before baking or frying, asparagine is converted to asparatic acid and ammonium. Because of this, asparagine cannot take part in the Maillard reaction, which greatly reduces the amount of acrylamide produced(16).

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ACRYLAMIDE:

Acrylamide, a chemical known to cause cancer in laboratory animals, develops during the high-temperature cooking of foods high in carbohydrates. Since acrylamide has not been found in foods that have been boiled or heated to a low temperature, it was thought to occur during high-temperature heating(17). This characteristic is associated with higher temperatures reached in Maillard non-enzymatic browning reactions, which are essential for the development of the ideal color, flavor, and aroma, particularly in the case of fried potatoes(18). When heated above 120 °C, some starch-based foods, including bread, French fries, potato chips, and processed cereals, can produce acrylamide(17).

APPLICATION OF L-ASPARAGINASE:

L-Asparaginase is an enzyme that plays a crucial role in pharmaceutical, drug development, agriculture, food industry and biotechnology applications(18,19).

PHARMACEUTICAL INDUSTRY:

- The main indication for L-asparaginase treatment is leukemia, specifically acute lymphoblastic leukemia (ALL).
- It functions by dissolving asparagine, an amino acid necessary for leukemia cells to grow and survive.
- By decreasing asparagine levels, it prevents the growth of cancer cells(19).

FOOD INDUSTRY:

- It is commercially significant to apply L-asparaginase in industrial processes to obtain a significant reduction in acrylamide in the finished product (like French fries).
- L-asparaginase has applications in the food industry to lower the production of acrylamide(20).
- Acrylamide is a potentially carcinogenic compound that develops when some foods are heated to high temperatures.
- L-asparaginase can be used to enzymatic ally degrade asparagine in food products, reducing acrylamide levels(21).

ANTIMICROBIAL ACTIVITIES:

- Industries are constantly looking for new L-asparaginase sources with improved yields and innovative properties. It has been claimed that the sources of this enzyme have antibacterial properties as well.
- To prevent contamination, this feature can be used in the bioprocessing of L-asparaginase. For the synthesis of this enzyme, marine sources are more varied and have received less attention(22,23).
- The bacteria from this source are expected to have unique characteristics since they can withstand a variety of environmental factors, such as temperature, pH, and salinity(22).
- The results of the other study reveal that pathogens like *Staphylococcus aureus, S. epidermidis*, *Micrococcus luteus*, *B. cereus*, *B. subtilis*, *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Aspergillus niger*, and *A. fumigates* are resistant to the antibacterial and antifungal properties of the leaves of *Amaranthus polygonoides*(24).

CLINICAL APPLICATION:
Anti-tumour agent:
- The enzyme L-asparaginase has been identified to be a clinically useful anticancer drug for the treatment of lymph sarcoma and acute lymphoblastic leukemia (ALL).
- For the development of tumour cells, L-asparaginase serves as a crucial amino acid(25).
- Since lymphatic tumor cells require a lot of asparagines to continue growing malignantly, giving them L-asparaginase causes the cells to become extremely low in asparagines, which renders the cells unable to sustaining.
- They use what they can generate themselves as well as the L-asparaginase in their food to meet their high asparagine needs(25,26).
- Therefore, while normal cell proliferation does not depend on the presence of L-asparaginase, it is essential for the development of tumour cells.
- The enzyme L-asparaginase provides tumor cells with a significant growth factor but also inhibits their ability to proliferate(27).
- These have powerful anticancer or anti leukemic effects(25).

MANUFACTURE OF FINE CHEMICALS:
- The production of fine chemicals depends heavily on the activity of asparaginases and L-glutaminases(28).
- Methionine, lysine, and threonine, which are members of the aspartic family of amino acids, are produced by L-asparaginase.
- Aspartic acid, which is thought to be a precursor to lysine and threonine, is produced when L-asparaginase breaks down asparagine.
- Utilising L-glutaminase to create -glutamyl alkamides is one of the industry's most important applications.
- In Japanese infused green tea, theanine stands out as a flavour-enhancing amino acid(29).

RESISTANCE TO L-ASPARAGINASE:
- By producing internal L-asparagine on their own, independent of normal cells, tumour cells develop resistance to L-asparaginase.
- Increasing the level of cytosine residue methylation in DNA, which is important for asparagine synthetase production, essentially allows for this to happen.
- Therefore, this enzyme boosts and controls the degree of L-asparagine production in cancerous cells. L-asparaginase resistance may also be caused by the higher amount of particular antibodies created to combat the accelerated rate of cleaved L-asparaginase.
- Reporters have asserted that patient immunisation had no impact on the drug's efficacy.
- Additionally, it has been observed that cells sensitive to L-asparaginase often release cytokines that manage and regulate the development of resistant cells.
- When the L-asparaginase kills the sensitive cells, the resistant cells escape the regulatory control and eventually expand to other organs.(30)

CONCLUSION:
In conclusion, this review paper has highlighted the significant role of L-asparaginase in the treatment of various cancers, particularly leukemia and lymphoma. It has discussed the mechanism of action, sources, applications, role in food industry, antimicrobial activities, Resistance to L-Asparaginase and Clinical application as Antitumor agent . L-asparaginase remains a crucial component of chemotherapy regimens, offering promising results in improving patient outcomes. However, ongoing research is essential to address challenges such as immunogenicity and resistance, ultimately optimizing its therapeutic potential.

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